GASTROINTESTINAL SYMPTOMS AND THE GUT IN AUTISM SPECTRUM DISORDERS: BURDEN, MEASUREMENT, AND ETIOLOGIC RELEVANCE

By

Calliope B. Holingue

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ABSTRACT

Background:

Individuals with ASD are more likely to experience gastrointestinal symptoms (GI). The goal of this dissertation was to shed light on the burden GI symptoms place on individuals with ASD and their families, improve the detection of GI symptoms, and assess the role of the gut as an etiologic risk factor for the incidence of ASD.

Methods:

The first study analyzed 12 qualitative interviews to summarize the experiences that families with a child with ASD and GI symptoms face. The second study reviewed the literature (n=144 studies between 1/1/1980 to 1/31/2017) on ASD and GI symptoms and described the range of approaches to ascertaining GI symptoms and conditions. The third study developed a questionnaire to assess GI symptoms in children with ASD and reported psychometric (n=537). The fourth study assessed for interaction between maternal immune activation and antibiotic use during pregnancy on the risk of ASD diagnosis in the offspring, in an enriched-risk prospective birth cohort (n= 142 ASD, 2,953 non-ASD).

Results:

Three themes emerged from qualitative interviews: Parents rely on behavioral indicators to detect GI symptoms in their child. GI symptoms are associated with poorer functioning and lower wellbeing and families tended to report negative experiences seeking healthcare for their child's GI symptoms. In the second study, GI symptoms were assessed many ways, and the assessment tool was significantly associated with symptom estimates. In the third study, we developed an internally consistent 35-item tool for assessing the presence of GI symptoms. In the fourth study, we found that the association between flu in the second trimester on risk for

ASD is modified by antibiotic use in pregnancy. Among women who did not receive an antibiotic during pregnancy, flu in the second trimester was associated with 4.4 times the odds of ASD diagnosis (OR=4.43 95% 1.13-14.69), but not among women who did receive an antibiotic (OR=1.06 95% 0.46-2.13).

Conclusions:

This work highlights the need for more work on the accurate measurement of GI symptoms in this population and the consideration of the gut as a risk factor for the development of ASD.

Academic Adviser:
M. Daniele Fallin, PhD, Professor and Chair, Department of Mental Health

Thesis Advisory Committee Members:
M. Daniele Fallin, Chair and Professor, Department of Mental Health (Adviser)
Li-Ching Lee, Associate Scientist, Department of Epidemiology
Noel Mueller, Assistant Professor, Department of Epidemiology
Sarah Murray, Assistant Professor, Department of Mental Health
Rashelle Musci, Assistant Professor, Department of Mental Health
Pankaj (Jay) Pasricha, Professor, School of Medicine

Dissertation Exam Committee Members:
Xiaobin Wang, Professor, Population, Family, and Reproductive Health (Exam Chair)
M. Daniele Fallin, Chair and Professor, Department of Mental Health (Adviser)
Li-Ching Lee, Associate Scientist, Department of Epidemiology
Sarah Murray, Assistant Professor, Department of Mental Health
Rashelle Musci, Assistant Professor, Department of Mental Health

Dissertation Exam Committee Alternates:
Joseph Bressler, Associate Professor, Environmental Health and Engineering
Heather Volk, Associate Professor, Department of Mental Health

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1.1.1 Autism history and core symptoms

Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder characterized by two core domains of deficits: social communication and impairment, and restricted, repetitive behaviors, interests, or activities[1]. Autism as a condition was first described in a case series of 11 children, published 1943 by Leo Kanner, a child psychiatrist at Johns Hopkins University, in his seminal paper, "Autistic Disturbances of Affective Conduct"[2]. The main features of autism, as described by Kanner, were the lack of affective contact with people, obsessive need for sameness, repetitive of verbal/motor behaviors, restrictive interests or obsessions with particular things, and communication impairments. Notably, Kanner emphasized the variability in cognitive impairments across the children he saw[2,3]. He wrote, "Even though most of these children were at one time or another looked upon as feebleminded, they are all unquestionably endowed with good cognitive potentialities[2]." Kanner's paper was followed a year later by a description of four boys of similar presentation by pediatrician Hans Asperger[4] in Germany. The term 'autistic' was in fact first coined by Swiss psychiatrist Eugen Bleuler in 1911 in his description of a symptom of severe schizophrenia[5]. Bleuler used 'autism' to describe the person's 'inner life', which was not observable to others. Specifically, Bleuler wrote that autistic thinking was concerned with childish/infantile desires to avoid the discomfort of reality and instead replace I with fantasies and hallucinations.

The field initially struggled with whether autism was a manifestation of childhood schizophrenia or its own diagnostic entity[6]. The conceptualization and classification of ASD has evolved since

it was first described. In the Diagnostic Statistical Manual (DSM-III-R) published in 1987, it was noted that the stereotyped, repetitive aspects of autism/pervasive developmental disorder could be confused for a delusion, but the additional diagnosis of schizophrenia should only be made in the rare case where 'prominent delusions or hallucinations' can be documented and meet the criteria for schizophrenia[7]. The DSM-III-R specified three domains of symptoms in autism: impairment in reciprocal social interaction, communication, and restricted and repetitive behaviors[7]. Waterhouse et al (1992) note that this criteria reflected Kanner's original description of autism[8].

In the DSM-IV and DSM-IV-R (1994 and 2000, respectively), Asperger's disorder, childhood disintegrate disorder, Rett's disorder, and pervasive developmental disorder not otherwise specified were added as 'Disorders Usually First Diagnosed in Infancy, Childhood, or Adolescence[9,10]." Debate followed regarding whether Asperger's disorder and Autism were distinct conditions. Ultimately, with the introduction of DSM-V, all conditions (autistic disorder, Asperger's, childhood disintegrate disorder, and PDD-NOS) were combined under one disorder: Autism Spectrum Disorder[1]. The decision to integrate Asperger's disorder into ASD stemmed from overlap in diagnostic criteria between the two disorders (Asperger's and autistic disorder) in DSM-IV[11]. Both autism and Asperger criteria included 'qualitative impairment in social interaction' and 'restricted repetitive and stereotyped patterns of behavior, interest and activities.' The key difference in diagnosis was the 'lack of delay or deviance in early language development' in Asperger's Disorder relative to autistic disorder[10,11]. In reality, however, both individuals with autism and Asperger typically had impairment in communication or abilities to sustain a conversation. The 'precedence rule' specified to only diagnose Asperger's disorder if

criteria for autistic disorder were not met; however, the overlap in criteria made this challenging[10–14]. The other main change that occurred in DSM-5 is that the three core domains of ASD where combined into two core domains: 1) social and communication impairments and 2) repetitive and restricted behaviors and interests[1].

1.1.2 Comorbidities in ASD

ASD is a clinically and etiologically heterogeneous disorder and is strongly associated with co-occurring psychiatric and medical conditions, which contribute to the burden of disease associated with ASD[15–18]. Children and youth with ASD are more likely to experience comorbid mental health conditions, including language disorders, tic disorders, attention-deficit hyperactivity disorder (ADHD), oppositional defiant disorder, conduct disorder, major depressive disorder, bipolar and related disorders, psychosis, separation anxiety disorder, specific phobias, generalized anxiety disorder, agoraphobia, social phobia, and obsessive-compulsive disorder (OCD). The exception is substance use disorders, which are less prevalent in individuals with ASD[16,19]. Further, children and youth with ASD are more likely to have physical health disorders, including epilepsy/seizure disorders, sleep disorders, gastrointestinal disorders, metabolic disorders, hormonal dysfunction, hydrocephalus, fetal alcohol syndrome and cerebral palsy[20,21]. The higher rates of these conditions contribute to greater health services utilization (though, interestingly, less preventive services), poorer quality of life, and increased burden and decreased wellbeing in the family[22–24].

Like children, adults with ASD also disproportionally experience mental and physical health conditions. They are at an increased risk for all major psychiatric disorders (with the exception of substance use disorders), including depression, anxiety, bipolar disorder, OCD, schizophrenia,

and suicide attempts. Medical conditions are also elevated compared to adults without ASD, including immune conditions, gastrointestinal disorders, sleep disorders, epilepsy/seizure disorders, obesity, dyslipidemia, hypertension, diabetes, and even some rare conditions such as stroke and Parkinson's disease[25,26]. A possible exception is cancer, which in some studies has been shown to be decreased in ASD, although the evidence is conflicting[25,27–29]. Importantly, the psychiatric and physical health conditions seen in ASD typically co-occur, meaning it is not uncommon for an individual with ASD to have multiple comorbidities[18].

1.1.3 Prevalence of ASD

The prevalence of ASD has increased dramatically over the last several decades. In the 1980s, ASD was believed to occur in 5 out of every 10,000 children (0.05%) in the United States (US)[30]. Data from 2012 indicated that 1 in 68 school-age children had ASD[31], while more recent 2014 data estimate prevalence among US children age 8 years to be 1 in 59[32]. Notably, the prevalence of ASD is 4-5 times higher in males relative to females[32]. This increasing prevalence has raised public concern and has turned attention towards possible explanations. It is very complicated to determine whether the increase in prevalence we've observed is in fact due to true risk factors. Research suggests that changes in diagnostic criteria, younger age at diagnosis, and increased ASD awareness in part contribute to the increased estimates[33–37]. However, as stated by Newschaffer et al., 'the question of whether this historical increase can be fully accounted for by these and other changes in diagnosis and classification remains open to debate, largely because it is very difficult to develop quantifiable estimates of diagnostic effects and virtually impossible to prove or disprove temporal changes in autism population risk profiles given the condition's unknown etiology[34].' Notably, there has not been a dramatic rise in the

prevalence of ASD with comorbid intellectual disability, suggesting that milder cases of ASD are responsible for the visible increase[38].

It's important to note that the prevalence estimates of ASD in the US vary by a number of demographic characteristics, especially race/ethnicity and socioeconomic status. Specifically, children who are Black, Hispanic, or of another race/ethnicity are less likely to receive a diagnosis of ASD compared to white children[39]. Critically, this variability does not reflect a biological difference in the risk of ASD but rather a disparity in access to diagnostic and other services. Communities with greater resources also have a higher prevalence of ASD diagnosis[36].

Estimates of ASD prevalence also vary across the world. Studies estimate that 0.6-0.8% of children globally have ASD, though we do not have data from many individual countries[40–42]. Registry based studies from Scandinavian countries estimate that 1-1.5% of children have ASD[43]. The study with the highest prevalence came out of South Korea and estimated that 2.64% of 7-12 year olds between the years of 2005-2009 had ASD[44].

1.1.4 Etiology of ASD

Twin and family studies support a strong genetic contribution to ASD, with estimates placing the heritability of ASD between 50% and 95%[45–47]. Correspondingly, one of the strongest risk factors for the development of ASD is having a sibling with ASD; studies suggest that the risk of autism varies from 3% to 18% in this population[47–49]. The genetic variants for ASD include both common variants with small effect sizes, and rare variants, including inherited and de novo mutations and copy number variations, that carry a larger ASD risk[50–53].

However, environmental factors also contribute to the risk of ASD, including advanced parental age[54–57], short (<12 months) as well as long (>60-84 month) inter pregnancy intervals[58–61], medication use[62–68], and pregnancy and birth complications, such as lower gestational age/preterm birth[69–72] , c-section[73], and metabolic conditions in the mother[74,75]. Environmental chemicals, such as air pollution[76–79] and endocrine-disrupting chemicals[80,81] have also been implicated in ASD risk.

Maternal immune activation has emerged as an important risk factor for ASD. Bacterial, viral, genitourinary infections, as well as fever during pregnancy have all been associated with increased risk for ASD in the child. The exact trimester in which the various MIA exposures influence ASD risk is not fully elucidated[82–88]. Further the understanding of how MIA exposures in pregnant women affect ASD risk in the child is an area of current investigation.

Critically, the interaction between a person's genetic makeup and exposure to environmental factors may influence their ASD risk. For example, there are instances in which an environmental stressor may only increase ASD risk in the context of a particular genetic variant[89–91]. Genetics and the environment also interact through epigenetics, which are heritable changes in gene expression that occur without any changes to the DNA sequence itself[92]. Epigenetics can be useful in ASD for a number of reasons: First, epigenetic marks might be involved in the etiology of ASD, by mediating the pathway between an environmental exposure and changes in gene expression. Further, epigenetic marks can be influenced by genetic

variation. Alternatively, an epigenetic mark can serve as a proxy or biomarker for a previous exposure or for a disease of interest[93,94].

1.1.4 Public health impact

ASD develops in childhood and tends to cause lifelong impairments. In addition to the core symptoms of ASD, medical and psychiatric comorbidities significantly increase the burden to the individual as well as their family/caregivers, and reduce the overall quality of life[16,24,95–98]. For these reasons, globally, ASD is among the leading causes of disability, in terms of years of life lost. In the year 2010, among children ages 5-14 years, ASD was the fourth leading cause of disability within the mental disorders. ASD also accounted for 7.7 million of disability-adjusted life years in the whole population[40]. Sadly, ASD is also associated with an increased risk of premature mortality[99–101]. The economic cost associated with ASD is also substantial. It's been estimated that in the US, the total annual costs of ASD are close to $250 billion and individual lifetime costs between $1.5-2.5 million[102]. Health care utilization costs contribute to these economic costs[103].

1.2 Gastrointestinal Symptoms in ASD

Gastrointestinal disorders are one of the most prevalent medical comorbidities in ASD, along with sleep disorders and seizures/epilepsy[21,104]. In Kanner's 1943 paper first describing autism, he wrote that 6 of the 11 children with autism "presented severe feeding difficulty from the beginning of life"[2].

Studies of individuals with ASD have consistently found elevated estimates of gastrointestinal (GI) symptoms, compared to individuals with typical development (TD) as well as those with other developmental delays (DD)[105–125]. There is no evidence of an ASD-specific gut pathology.

Rather, individuals with ASD appear to be an elevated risk of GI symptoms and disorders[107]. The most common GI symptoms found in ASD are chronic constipation, diarrhea, or alternating constipation/diarrhea, abdominal pain, and acid reflux, though other disorders are elevated as well[107,126]. Notably, GI symptom estimates have varied considerably across studies, likely due to the heterogeneity of ASD as well as the lack of standardized and validated GI symptom assessment tools for ASD, which will be discussed in greater depth below.

Gastrointestinal symptoms are distressing not only because of the pain, discomfort, and functional limitations, but also because of their effect on mental and physical health. Individuals with ASD and GI symptoms are more likely to have sleep disruptions[127,128], aggressive, irritability, externalizing, or self-injurious behaviors[128–132], anxiety and mood problems[131–133], sensory sensitivities/over-responsiveness[133], toileting problems such as soiling[134], food sensitivities and eating issues[128,135–137], and other psychopathology and somatic issues[131,138,139]. The high prevalence of GI symptoms in this population has contributed to the popularity of complementary and alternative treatments or medicines (CAMS), which are therapies developed outside of conventional Western medicine that are either used with (complementary) or in the place of (alternative) conventional medicine[140]. Families with a child with ASD may turn to CAMs when western medical approaches have failed them[141–144]. With respect to ASD, biological therapies (e.g. special diets, nutritional supplements, detoxification, chelation) in particular, are often touted as ways to heal the gut and therefore cure autism[145]. Unfortunately, there is little data on the safety and efficacy of such CAMs[141–144]. Further research needs to be done to understand the benefits and risks of these CAMs and their interaction with the gastrointestinal system.

1.3 Explanations for Association between ASD and Gastrointestinal Symptoms

There are a number of possible explanations for the association between the gut/GI symptoms and ASD. The bidirectional communication that occurs between the brain and the gastrointestinal tract is broadly referred to as the "gut-brain axis"[146–149]. The gut-brain axis is mediated through a number of pathways: endocrine (e.g. cortisol), immune, neural (vagus nerve and enteric nervous system), neurotransmitters produced in the gut (serotonin and tryptophan), production of short chain fatty acids, and the gut microbiome, which may interact with the other pathways[149].

Gastrointestinal symptoms in ASD may reflect dysbiosis, or imbalance, of the gut microbiome. The human microbiome, comprising trillions of bacteria and other microorganisms, coexists with the human body and is involved in critical functions such as digestion and stimulation of the immune system[150,151]. The microbiome is dynamic across the life course, influenced by early life exposures, highly variable across body sites within a person, and has been associated with a number of diseases including gastrointestinal cancers, inflammatory bowel disease, type 1 diabetes, rheumatoid arthritis and multiple sclerosis[150,151]. Gut microbes are known to influence intestinal barrier integrity, epithelial cell proliferation, mucus production, and GI motility[152–154]. There is also growing evidence that the gut microbiome plays a significant role in immune dysregulation, which is also a risk factor in ASD[155].

Existing studies have found that individuals with ASD have increased relative abundance of Clostridium bacteria[156–158], Sutterella[159,160], Lactobacillus and Desulfovibrio[161], and decreased

relative abundance of Prevotella, Coprococcus, and Veillonellaceae[162,163], relative to people without ASD. A decreased Bacteroidetes/Firmicutes ratio has also been linked to ASD[161]. However, findings have been inconsistent across studies[164]. Few behavioral traits or co-occurring issues in ASD have been examined for associations with the gut microbiome. Kang et al. found that among children with ASD, ASD severity, GI symptom severity, being on a gluten-free/casein-free diet, consumption of probiotics, nutritional supplements, eating too much, or having an extremely limited diet were not significantly associated with the top 10 genera found in the ASD group[162]. However, other co-occurring issues, including sensory sensitivities, and medical and psychiatric comorbidities have not been examined for associations with the gut microbiome among individuals with ASD.

Further evidence for the role of the gut microbiome in causing or influencing ASD symptoms is provided by a recent open-label trial which found that a 2-week antibiotic treatment, bowel cleanse, and extended fecal microbiota transplant led to reductions in GI and ASD symptoms[165]. However, this study was not blinded and did not have a placebo-controlled ASD group. It is also not clear from this work whether improvements in ASD behavioral symptoms were due to biological changes in the gut microbiota, or due to improvements in GI, which in turn improved the participants' overall wellness.

Animal literature thus far provides the best evidence for the importance of the gut and gut microbiome in influencing neurodevelopment and possibly causing ASD. A 2017 study by Kim et al. demonstrated that in a mouse model, the composition of the maternal gut microbiome modifies the effect of maternal immune activation on the developing brain. Treatment with a broad-spectrum antibiotic suppressed differentiation of Th17 Cells and ultimately secretion of

IL-17a, by wiping out bacteria that are responsible for promoting this inflammatory response. This in turn prevented development of ASD-like behavioral symptoms and cortical patches in the offspring, which typically occurs in this maternal immune activation mouse model[166] (see Figure 1a & b). T1his work has since been replicated by others[167]. This research highlights the potential for the maternal gut microbiome and early-life infant gut microbiota to have critical effects on neurodevelopment, including incidence of ASD.

Figure 1a. Maternal immune activation leads to neurobehavioral abnormalities and cortical patches in mice offspring (Kim et al. 2017)

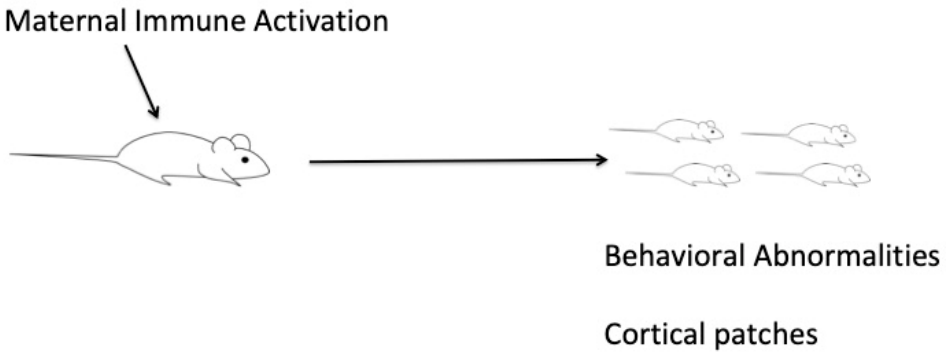
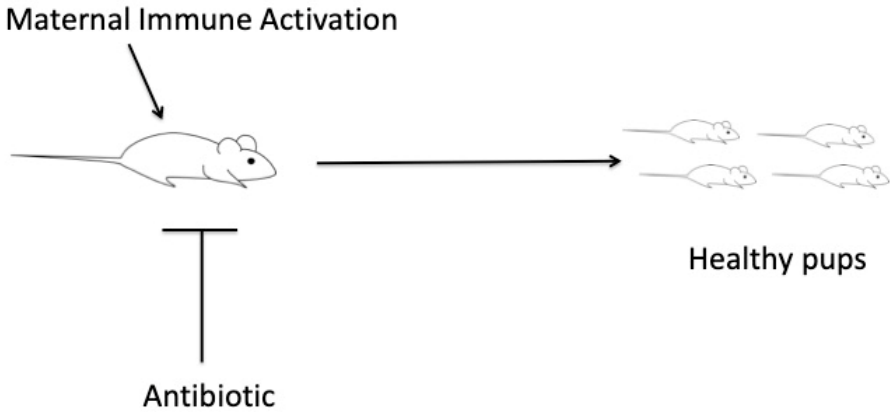


Figure 1b. Administration of antibiotic blocks the effect of maternal immune activation, rescuing the neurodevelopmental abnormalities and cortical patches (Kim et al. 2017).



Another way in which GI symptoms may be linked to ASD is through the behavioral consequences of having significant GI distress. Gastrointestinal symptoms are likely to cause or exacerbate behaviors such as irritability, sensory sensitivities, anxiety, aggression, or even self-injurious behaviors, particularly in an individual who is not able to communicate regarding the presence or nature of their GI symptoms. Behaviors that are typically ascribed to ASD may sometimes may manifestations of underlying medical distress[107]. Unfortunately, diagnostic overshadowing, or the process of misattributing symptoms to a mental disorder, can lead to these GI symptoms being under recognized and therefore undertreated[168].

Finally, having ASD may be a risk factor for the development of GI symptoms. People with ASD are more likely to have a restricted, particular diet (e.g. avoiding entire food groups, eating only certain colors food, having dietary allergies or sensitivities) which can affect the health of their gastrointestinal system and lead to symptoms such as diarrhea, constipation, and abdominal pain[135,169]. The presence of physical and mental health comorbidities, such as anxiety, depression, or seizures, and the medications taken for those conditions, may also affect the gastrointestinal system[133].

The various pathways by which ASD and GI symptoms/the gut may be associated are depicted in Figure 2a & 2b.

Figure 2a. Pathways by which ASD may lead to GI symptoms.

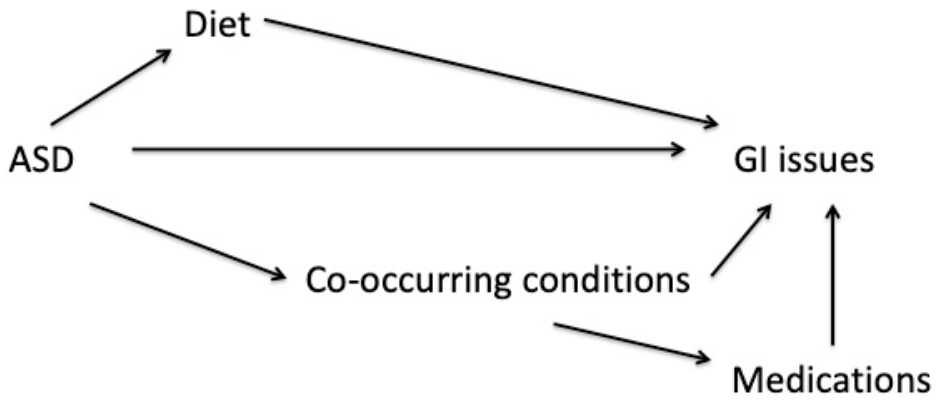
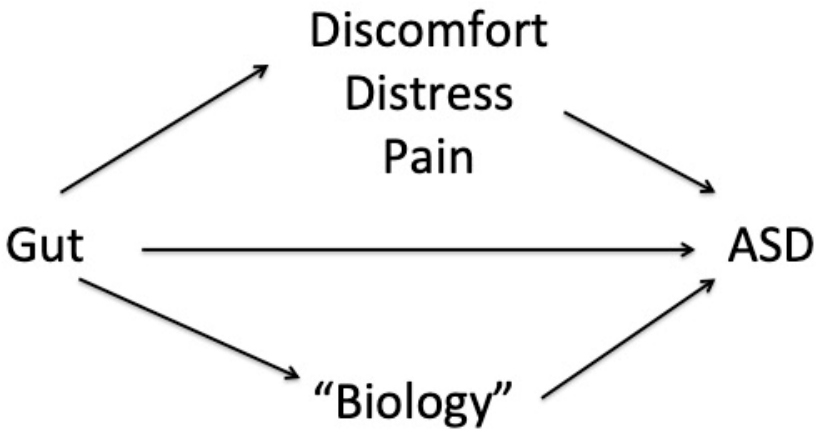


Figure 2b. Pathways by which the gut may lead to the development of ASD or comorbid behaviors in ASD.



1.4 Measurement of GI symptoms in ASD

A serious obstacle to carrying out rigorous research on the gut-brain connection in autism is the lack of reliable and valid tools for the assessment of GI symptoms. Because children with ASD in particular may have limited ability to self-report due to communication impairments, traditional approaches that query GI symptoms and distress may misclassify persons. Valid tools for detecting GI symptoms in ASD will likely include behavioral indicators. The importance of these types of indicators has previously been outlined, including in a 2010 consensus report by Buie et al. titled 'Evaluation, diagnosis, and treatment of gastrointestinal disorders in individuals with ASDs: a consensus report[107].'

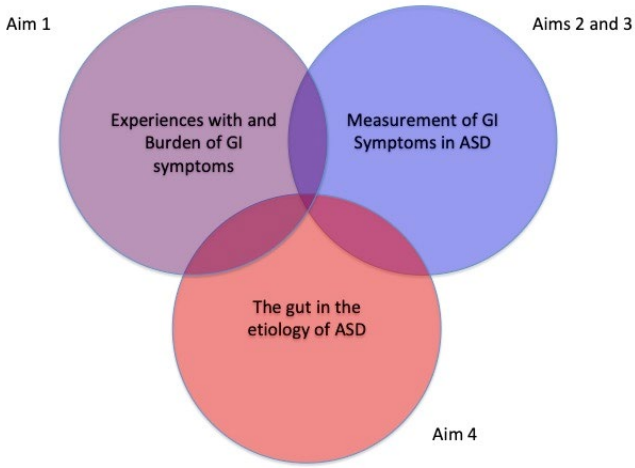
Currently, one single tool for assessing gastrointestinal symptoms in individuals with ASD has been assessed for psychometric performance[126]. While the development and assessment of this tool meets a critical need in the field, limitations to this tool remain, including the limited assessment of dietary and mealtime factors, which are often related to gastrointestinal symptoms in individuals with ASD. A reliable and valid questionnaire would enable comparisons of symptom estimates across studies, over time, following interventions, and with respect to risk factors. Development of a standardized, reliable, and valid questionnaire will help reduce the influence of noise and bias on GI symptom estimates in observational and experimental studies. The field can then begin to understand what role the gut plays in ASD, if and how GI symptoms help distinguish between subgroups of individuals with ASD, and how these subgroups differ in terms of comorbidities and trajectories.

Clinically, it is also important to be able to better detect when individuals with ASD have GI symptoms. In individuals who have difficulty communicating GI symptoms/distress to their parents/caregiver or healthcare providers, gastrointestinal disorders may go undetected and therefore untreated, exacerbating the distress for the person with ASD[107], and leading to medical and psychiatric comorbidities. Being able to prevent or treat gastrointestinal symptoms can significantly improve the quality of life of someone with ASD.

1.5 Statement of Problem and Motivation for Research

Despite decades of evidence showing that GI symptoms are elevated in individuals with ASD, our understanding of the role of these GI symptoms, and the gut, in ASD is still in its infancy. Further, our ability to accurately measure GI symptoms and our understanding of how these symptoms manifest and affect the functioning of individuals with ASD is also limited. In this dissertation, I took a tripartite approach to studying the gastrointestinal system/gut in ASD (Figure 3).

Figure 3. Tripartite Approach to the Gastrointestinal System/Gut in ASD



First, I sought to better understand the experiences with and burden of GI symptoms in individuals with

ASD and their families. Second, given the current state of measurement of GI symptoms among individuals with ASD, I worked to develop and evaluate a better GI symptoms questionnaire tool for use in research, and potentially also clinically. Lastly, I pursued further evidence for a direct relationship between the gut and the etiology of ASD.

The first motivation for this dissertation is that GI symptoms in ASD may significantly impact functioning of the child as well as the family, as well as increasing the risk for other co-occurring issues including behavioral problems, sleep problems, and psychopathology. Sadly, less attention is paid to GI symptoms, as well as other comorbidities, because they are often attributed to the child having autism. Therefore, individuals with ASD and GI symptoms typically do not receive the same evaluation and treatment as typically developing individuals. Further, ASD-specific issues such as communication impairment and, restrictive and repetitive behaviors, sensory sensitivities and aversions, and strong dietary reactions or sensitivities, may interact with the presentation, development, and impact of the GI symptoms. Given this context, GI symptoms can significantly decrease the quality of life of a child with ASD. The goal of Aim 1 was to gain a deeper understanding of the issues children with ASD and their families face in regards to GI symptoms and to describe how GI symptoms can manifest in this population. The qualitative data from this aim highlights the burden that GI and related issues face on individuals with ASD as well as their families, and emphasizes the importance of further work in this area.

Next, because of the challenges associated with measurement, GI symptoms in individuals with ASD are more likely to go unrecognized. Physicians are less likely to pursue full diagnostic assessments, and therefore individuals with ASD are less likely to receive treatment and the

same quality of care as their typically developing counterparts. Further, the epidemiologic study of ASD, as it pertains to GI symptoms and the gut, is complicated and potentially biased. In order to accurately assess the role that GI symptoms and the gut plays in 1) causing ASD, 2) influencing the safety, efficacy, and effectiveness of interventions/treatments, 3) responding to interventions, including CAMs, 4) and affecting and being influenced by co-occurring issues, we need to improve our assessment of GI symptoms. This is the motivation for Aims 2 and 3. The goal of Aim 2 is to describe the range of approaches to ascertaining GI symptoms and conditions in studies of ASD, and to assess how the variation in measurement approach is associated with GI symptom prevalence estimates. The goal of Aim 3 is to develop a reliable and valid questionnaire that assesses gastrointestinal (GI) symptoms in children with autism spectrum disorder for use in epidemiologic studies.

The last motivation for this dissertation is the possibility of the GI system/gut playing a role in the etiology of ASD. Prior human and animal literature has documented differences in the gut microbiome of children with ASD, and has implicated early-life infections and maternal immune activation as risk factors for the development of ASD. Recent animal work has shown that administration of an antibiotic (through affecting the composition of the maternal gut microbiota) at the time of MIA blocked the neurodevelopmental abnormalities that are typically seen in the context of MIA. This has not been tested in humans however. The goal of Aim 4 is to assess for interaction between maternal immune activation and antibiotic use during pregnancy on the subsequent risk of ASD diagnosis in the offspring, within an enriched-risk prospective birth cohort.

1.6 Dissertation Aims and Outline

This dissertation is organized into 6 chapters. This introductory chapter provides an overview of the rationale for examining the link between the gut and ASD and provides an outline of the specific aims of this dissertation.

In Chapter 2, the results of the Aim 1 qualitative study are presented. I summarized the experiences of parents with a child with ASD and GI symptoms, describe how GI symptoms can manifest in this population, and highlight the burden that GI symptoms place in children with ASD.

Chapter 3 describes the results of Aim 2 study, a literature review to describe the range of approaches to ascertaining GI symptoms and conditions in studies of ASD, and to assess how the variation in measurement approach is associated with GI symptom prevalence estimates. Epidemiologic studies of ASD including information on gastrointestinal symptoms/disorders were reviewed (years 1980-2017). Recommendations were outlined for the critical components needed in a reliable and valid GI questionnaire.

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Chapter 4 describes the results for Aim 3, development of a reliable and valid questionnaire that assesses GI symptoms in children with ASD, for use in epidemiologic studies. I developed this questionnaire using two existing tools as well as new items, which I derived from the extant literature, an expert panel, and qualitative interviews with parents of children with ASD and co-occurring GI symptoms. I administered this questionnaire along with the Child Behavior Checklist (CBCL) to an online research registry of parents who have a child with ASD between

the ages of 3 and 17 years old. I tested the questionnaire for reliability and validity and reported the psychometric characteristics.

Chapter 5 describes results for Aim 4, assessing for interaction between maternal immune activation and antibiotic use during pregnancy on the subsequent risk of ASD diagnosis in the offspring, within an enriched-risk prospective birth cohort using electronic medical record data, extracted medical chart information, and a maternal postpartum questionnaire.

Lastly, in Chapter 6, I summarize and synthesize the results of all these studies, discuss the implications of this work, and outline the next steps for research in this area. The ultimate goal of this work is to improve the detection of GI symptoms in individuals with ASD, assess the role of the gut as a causal risk factor for the incidence of ASD, and to decrease the burden that GI symptoms place on individuals with ASD and their families.

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CHAPTER 2: A QUALITATIVE STUDY OF FAMILY EXPERIENCES WITH HAVING A CHILD WITH AUTISM AND CO-OCCURING GASTROINTESTINAL SYMPTOMS

Coauthors: Sarah Murray[1], Dani Fallin[1,2]

[1]Department of Mental Health, Johns Hopkins Bloomberg School of Public Health; [2]Wendy Klag Center for Autism and Developmental Disabilities, Johns Hopkins Bloomberg School of Public Health

2.1 Background

Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder consisting of two core domains of symptoms: social communication and impairment, and restricted, repetitive behaviors, interests, or activities[1]. Psychiatric and medical comorbidities are very common and contribute to the burden associated with having ASD[2–4].

Gastrointestinal symptoms, in particular, are prevalent among individuals with ASD; common symptoms include constipation, diarrhea, and abdominal pain[5–26]. These GI symptoms typically co-occur with feeding issues including strong dietary preferences and sensitivities or allergies, as well as toileting problems such as delayed potty training, encopresis, and wetting the bed. Individuals with ASD who have GI symptoms are more likely to exhibit aggressive, irritable, externalizing, and self-injurious behavior; experience anxiety and mood problems; and, have other co-occurring psychological or somatic conditions[27–37].

Despite the high prevalence of GI symptoms in this population, there has been little research aimed at understanding the experiences of children with GI symptoms and pain or the resulting challenges experienced within families. Sensory issues, impairments in communication, and restrictive and repetitive interests (including strong food preferences) common to ASD may interact with the GI symptoms to introduce new challenges and considerations to the evaluation, management, and burden in comparison to typically developing populations. Thus while GI issues are associated with chronic pain and both psychiatric and non-psychiatric comorbidities[38–40] in general child populations as well, research done with typically developing samples of children may not generalize or be applicable to children living with ASD.

Qualitative research is a valuable tool for identifying the scope of an issue, capturing the complexities and nuances of human experience, and identifying targets for intervention [41]. While prior qualitative research has examined food sensitivities, picky eating behavior, and other feeding challenges among children with ASD, these studies have only peripherally explored GI symptoms as co-occurring issues[42–46]. To address this gap in knowledge, we conducted semi-structured interviews to explore parents' perceptions of their and their children's experiences of gastrointestinal symptoms while living with ASD. Specifically, we sought to understand how parents identify when their child is having GI symptoms or distress to aid in the development of a GI questionnaire for children with ASD (as discussed in Chapter 4). Secondly, we wanted to gain a deeper understanding of the issues families and individuals with ASD face regarding GI symptoms, such as how it affects family functioning.

2.2 Methods

2.2.1 Participants

One-on-one semi-structured interviews were conducted with parents of children with ASD. Parents were also given the option to have their child join the interview. Participants were eligible if they were a parent or primary caregiver of a child with an ASD diagnosis who experienced GI symptoms anytime from the age of 3 to 17. These interviews were initially designed to aid in the development of the item pool for a GI questionnaire for children with ASD (Chapter 4). Social media outlets (e.g. Facebook), email listservs, and website postings were used to recruit individuals from advocacy groups and other ASD-centered groups. Participants

received a $10 Amazon gift card for participating, as well as access to a free webinar for study participants describing study findings.

2.2.2 Data Collection

Interviews were conducted by the first author and took place either in person in a private location convenient to the participant (e.g. the participant's home) or remotely via Zoom Video Conferencing Platform. Interviews lasted approximately 30-45 minutes on average. All interviews were audio-recorded with permission and transcribed, incorporating notes taken by the interviewer. An interview guide was used that included the following key questions:

1. What are the gastrointestinal issues your child currently struggles with or has struggled with in the past?
2. What are things you notice about your child when they are having GI symptoms/distress?
3. What are some signs/behaviors that you see?
4. What areas related to GI issues have affected your family or your child's functioning?

We also asked follow-up questions and probed answers for more details. Given the semi-structure nature of the interviews, the exact language was adapted to each participant and simpler language was used when interviewing a child or person with cognitive impairment.

2.2.3 Analysis

The first author re-read all transcripts prior to analysis to familiarize herself with the whole of the data. A directed content analysis was then conducted using the core questions to partially inform the identification of themes across interviews following a general inductive approach[47,48]. We assigned quotes from the transcripts to themes. References to potentially identifying information

were removed from any quoted text to preserve the anonymity of participants. Because dietary issues by themselves have been thoroughly outlined in prior literature, we focused on themes that had to do with non-dietary issues.

2.2.4. Ethical Considerations

The local institutional review board approved this study. Informed consent was obtained from all parent participants prior to initiation of the interview. To be present and participate in the interview with their parent, participants with ASD younger than 18 provided assent in addition to their parent providing permission. Participants with ASD age 18 or older who participated with their parents consented for themselves, unless the parent or primary caregiver deemed their adult child unable to consent, in which case we relied on parental permission and assent as per the procedures for children under 18 with ASD.

2.3 Results

2.3.1 Participant Characteristics

In total, twelve qualitative interviews were conducted: ten with mothers of a child with ASD and two with fathers. Only two of the children with ASD participated alongside with their parent in the interview. All but one parent was speaking about a child with ASD who was male, and the age range of the children with ASD at the time of the interview was 5-25 years old. Nine of the interviews were in reference to children less than 18, though all of our interviews asked about GI symptoms in childhood specifically. Participants lived in the Baltimore/Washington D.C. area.

2.3.2 Thematic Analysis

Three overarching thematic categories emerged from the data: 1) indicators of GI symptoms/pain in children with ASD, 2) impact of GI issues on individual and family functioning, and 3) experiences with the healthcare system related to children's GI symptoms. Below we summarize each theme and provide illustrative quotes.

Theme 1: Indicators of GI symptoms/pain in children with ASD

Most parents reported that their child did not directly, verbally communicate that they were experiencing GI symptoms. Rather, parents reported needing to rely on indicators or signals to identify when their child was in pain.

> *"*...It's hard you know, children, people with autism in general, [child name] does, I noticed others have a very high pain tolerance. It's exceptionally high…he's more crabby or more irritable, more demanding. Those are days that you would assume that he's just not feeling right…he's nonverbal and it's hard…it's like communicating with an 18 month old."

This was often the case even for parents of high-functioning, verbal children, who still were described as having difficulties expressing or describing pain to their parents verbally. Sometimes, this might be that children could indicate the experience of some pain, but not fully describe what was going on to the point the parent could understand. In other words, children themselves had difficulty understanding the nature of the symptoms or trouble communicating to their parent the type of GI symptoms they were experiencing.

*"*He is verbal to the extent where he can talk to you about things but when something about his body or his feelings or anything that makes him uncomfortable, he doesn't have any words for it"

"A lot of it felt like needles almost...Other times it just felt like pushing…outward kind of…" [Child with ASD describing GI symptoms]

"He gets angry. Short, kind of semi belligerent with really basic questions, very atypical for him on a daily basis and then find out that he had just had to go to the bathroom. That's how he kind of presents. But then if you ask him, "Do you need to go to the bathroom"…'No, I'm fine.'"

Parents discussed a variety of behaviors they observed in their child that alerted them to the possibility of GI symptoms. These behaviors ranged from non-GI specific, e.g. irritability or sleep issues, to aggression. Parents explained that they learned to identify these behaviors through trial and error, particularly by noting improvement in the behavior after the child's GI symptoms improved. For example, as one parent described:

*"*When he was feeling hurt [with gas in his belly] he wasn't just crying, but like kind of had this pleading look on this face…kind of like surprise and shock…I just was able to draw the connection because when I would rock him like that or soak him…he would pass the gas. But there wasn't any trick, like he wasn't rubbing his tummy or he wasn't like pointing to it or you know…the only way I knew is probably trial and error."

*"*My very first cue was difficulties sleeping…He was upset…really upset…He was hardly sleeping…"

In some cases, the presence of GI symptoms was more obvious, because of symptoms such as bloating or gas.

> *"*...I think he would even act like his stomach hurt him, like you know holding his stomach…His stomach was bloated, like his stomach was very full always…He always had this big round the belly. And he might even have said his stomach hurt from time to time. He would complain of like 'my tummy hurts.'"

> *"*Well he had them [GI symptoms] starting as a baby. And so I mean something as simple as putting my hand on his stomach, I could feel everything moving and growling...he would pull his legs up and scream. I mean he had severe gas. They tried to work on a lot of that to no real avail. And so you could you could see it in his movements…Sometimes I would have to bring him to the doctor and they would basically have to assist because it would become so dried out [stool] that it would no longer move through his system."

Parents shared that in some cases of extreme GI distress; their child would act very aggressively or violently, either to themselves or to others.

> "…When the police had to come because she was destroying a lot, she would tell them 'my tummy hurts'...anxiety seems to have a direct reaction. Whatever it is that is upsetting her seems to manifest itself in her stomach. And that's when she acts out…. When the stomach hurts, there's an almost immediate physical reaction. She could scream, she could throw things. And after she calms down then she tells us 'My tummy

hurts'. Every time the police had to come out because she was really being destructive, first thing she would say as she regained her composure was 'my tummy hurts'.

"The only way I can really tell to be honest is that his behavior just is very radically aggressive and violent. Or if he doesn't want to sit down."

"...When my tummy hurts I hit my head." [Child with ASD]

"...He has [number of siblings]…when he is violent when he's constipated, I cannot always protect them. I had to teach them, if he is looking like he's going to hurt them they need to get away and be safe. Because at school he has many more people to his one's self than I am…

Lastly, a child's prior experiences with GI symptoms were described as contributing or potentially exacerbating future symptoms. For example, one parent noted "…withholding the stool I think is because he must have gotten constipated and then had a painful stool one time."

Theme 2: The impact of GI issues on functioning among children with ASD and their family

Parents shared that the child's GI symptoms affected multiple domains of the child's life, as well as the family's overall functioning and wellbeing. One of the major domains of life affected by the child's GI symptoms was his or her ability to attend and stay in school. Parents noted challenges with getting their child out the door in the morning because of toileting issues.

"It affects the ability for him to get out of the house and leave in the morning. So it was very stressful for him and for us you know you get up early and it might take him…two

> hours or one day it might be right away and another day is you know three hours or …gets there [school] and he's in the bathroom. And there are definitely behaviors associated with the frustration of having stomach pain and having nobody be able to fix that.*"*

In addition, parents mentioned having to pick up their child from school because of GI symptoms or accidents. For example:

> "School will call and just say things like 'you know he just doesn't feel well today. He's been in the bathroom a lot too.' And I just throw in the towel and I go get him. That's all you have to say, we're going to come get him."

One family noted that their choice of school was influenced by the presence of their child's GI symptoms.

> "…He ended up in a special school… because his combination of disabilities including autism was so severe that…our school system did not have a particular program for him. And the gastrointestinal actually was one of the major problems they were having because you know they did not have a one-on-one to sit in the bathroom with him while he had to sit there for 30 minutes or more. So they decided he needed to be in a special school."

In addition, while at school, children's' GI symptoms were described as affecting their ability to focus and learn.

*"*It will inhibit his ability to go out and do things sometimes because he can't stay off the toilet all day. It affects his behaviors...I think it affects his ability to learn at school because he feels so rotten."

*"*When he is not right in his gut, He is not right and the whole world isn't right… now that I think about it a lot of his behavior and his issues really crop up when he is constipated and he will get in trouble more. He will lose privileges. He will get low point chart numbers from school. So I mean it impacts his daily life because of his behavior changes. And he's also sometimes afraid of going to the bathroom at school because he knows that sometimes his bowel movements are rather large and he doesn't not want to clog the potty. And he has had the janitor have to come and unclog it and I think that was really, really embarrassing"

Children also expressed to their parents embarrassment about going to school having GI symptoms. One parent stated that at school their child 'might really need to go to the bathroom and maybe there's a way that …someone could go and be his bathroom buddy or something. Just to make it more comfortable for him."

Aside from school, GI symptoms also affected the child's likelihood to engage in social or extracurricular activities.

"...On good days she enjoyed going out and participating. On days where her tummy hurts sometimes she doesn't even want to go out."

"…You will observe certain days that you know he's just not into his preferred activities. He'd rather lay down…"

Parents reported that the entire wellbeing of the household was affected by the child's GI symptoms. The severe pain and distress that accompanies severe GI issues causes not only pain for the child with ASD, but also pain and trauma for the family overall.

> *"* Well with the constipation…he'll start screaming or start crying or start breaking out into a sweat. Yeah I mean he's just inconsolable in pain. I sort of figured out how to get him through it. There is only one time where I was like …'Do I have to call the emergency room?' But every other time it's kind of taken about 30 to 45 minutes where we figured out a way to get it out of him…. [he is] a wreck…crawled up into a ball and it's just awful...it's traumatic for everybody."

> "…I cannot always give him the support he needs…so it hugely impacts what kind of temperament the household has."

> "…Trying to administer things like suppositories or things that we know he didn't like…it's painful as a parent to have to try and do something that's uncomfortable or out of the norm to your child just because you know they don't really care for it"

> *"*...Not only are we trying to deal with him and his problems and you know come up with new foods and eliminate … but we're also arguing with everybody… the school…kind of arguing with the doctors who think we're exaggerating…we're arguing with family members who are thinking you're just being you know helicopter parents ...ruins our whole family life but also disturbs his sleep. You know everything. So you know it puts a lot more tension on the entire family."

A particular area that was affected by the child's GI symptoms was the ability to go out as a family, because of symptoms like diarrhea, or because of the child's special dietary needs or strong food preferences.

> "The sensory issues around eating used to stress us out like crazy…it's really hard to go out to restaurants with him because it just depends on his mood. And sometimes Mommy doesn't want to cook. We want to go out and treat ourselves or something…We kind of figured out what time of best, and what type of restaurant is best as far as not being too loud and noisy...Puts a damper on everybody's well-being when you can't always eat out and we have to factor in the wait time for getting seated and for how long it's going to take to get the food on the table."

> "…If he really has you know the diarrhea we you know we just can't go anywhere. And he won't wear a pull up…. a lot revolves around him in our house. Around his toileting."

> "…You know wanting to not go anywhere or do anything until we could resolve it…"

Lastly, parents also reported financial stress associated with the child's GI symptoms or noted spending a lot of money on GI-related interventions.

> "…He's almost 10 now. I don't have the resources anymore to go see the doctors that don't take insurance and to try the therapies my insurance isn't going to cover…we're still paying for all of the other stuff we did. And I can continue to dig our family into a hole which causes a different kind of stress…There's almost an acceptance sort of issue happening now…Do we just have to accept that this is the way things are going to be for him…"

"Oh God. We spent so much money on probiotics."

Theme 3: Experiences with the healthcare system.

The last core theme identified from the interviews related to the experience of families with the healthcare system regarding their child's GI issues. While some parents reported satisfaction with a particular provider or health care setting ("…Liked them both... I think they were really good at what they do…"), parents tended to have negative experiences when seeking medical help for their child's GI symptoms.

One of the frustrations parents had was with the long wait time between making an appointment and seeing a provider, and relatedly, with the shortage of providers.

> "The time lag is horrendous. I don't think there are enough GI doctors who get this…So that's my frustration is that the lag time…it takes, you know, weeks to get the appointment."

One parent noted that these long 'lag times' play a role in the promotion of complementary and alternative medicine use for GI symptoms among families with a child with ASD. Another strategy to cope with these lags included the sharing of information among parents:

> "...So there's a lot of a lot of parents who [say] 'This happened to me and this is what I did. Maybe try it because like how bad could it be.' To have them do a cleansing and see if a reset helps. I mean you don't really need a physician for that. Check with your

pediatrician; see if it's OK. So there's a lot of parent advocacy and sharing of information because the lag is bad."

Parents were also frustrated with financial obstacles, and in particular, insurance companies and the many steps it takes to get approval to see a provider.

"A lot of it is the insurance company. Because so many things of what you do with these children are not covered by insurance. Providers don't take insurance, you have to bill it on your own. And the insurance company is a huge aspect in all of it…"

"I guess there's financial obstacles that a lot of the treatments are not necessarily traditional treatments and would be outside of the scope of your traditional health care coverage. So basically you would generally have to go out of pocket because it's not going to be approved under your typical medical ...There's usually 30 steps you go through…[to] get a referral."

Another challenge in seeking healthcare is that office setting environments in general are not generally accommodating to children with ASD and staff and medical professionals are not trained in how to care for these children.

"A lot can be attributed to the setting of offices. A lot of these gastrointestinal and any other physicians that we've seen they have these huge practices. The waiting rooms are loud and noisy and full of fluorescent light...just being in the building is hard. "

*"*There's sensory issues, there's medical and there's practical issues that you know it's dragging a 6 year old with severe autism to doctors appointment after doctors appointment. They don't sit quietly…it's hard to do."

"I've brought him places and they have looked at his behavior just as we walked in the door and said you know 'we cannot provide service.' He's throwing furniture in doctors offices...If the waiting rooms weren't so big and so crowded and it didn't take so long to get in and the little room didn't take so long to see the doctor…I feel like you know all of the courage that he brings to the doctor's office wears off by the time the doctor gets there."

An additional challenge for the parents was how to physically get the child to the office visit.

"…It's driving a six year old son with severe autism to do it…and then when you go to these places and have to go somewhere that they've never been before and they're not happy about it and then you're waiting an hour…just the emotional physical toll it takes on you. To what end. And then you know then what do they need…they need blood work and I don't know if you're familiar with what it's like to get blood from a child severe autism but…you would literally need to gas my child in order to get blood. So it's like what what's the point… if there's just so little in the way of what can be done."

Parents shared strategies that they used to cope with the environmental challenges associated with these GI appointments. For example, one parent stated, "…You need to have the ability to maybe have a quieter space to get to prep your kid. They need to prep the hospital and the

nurses like ‘this isn't going to be like your regular 15 year old’. You know we need to do a little sedating on the way…”

Lastly, a critical concern is that parents felt they were not taken seriously due to their child having ASD. Further, they felt that providers were not used to seeing complex medical cases like the ones their children have. Parents expressed wanting to find someone who is curious enough to dig deeper and help them find solutions for their child.

> “A lot of physicians seem uninterested...It's sort of like ‘your child has autism. This goes with it.’”

> *“*…It feels like we have to push to even get them [the physicians] to test…”

> *“* ... I haven't found anyone yet who curious enough yet to be willing to dig a little deeper...There are times I wish I hadn't told them he had autism.”

> *“*I just want someone to care. I just want someone to like look me in the eye and say ‘I'll help you find it’…and I haven't found that person yet.”

> *“*I think that some of the issues that happen are more complex and they are expecting a child to come in with a fever and you know figure out the cause of that fever and whether or not they require medication. And that's the end of it. We have a lot of ongoing issues and things that may affect other things and it's just more complex.”

2.4 Discussion

In this qualitative study, we explored three key categories of experiences related to GI symptoms experienced by children with ASD: 1) indicators of GI symptoms, 2) impact of GI symptoms on the child's and the family's functioning, and 3) experiences with the healthcare system.

Related to how parents learned or could identify that their child with ASD had GI symptoms, typically this was not through child self-report, either because of general communication deficits or an inability to describe what symptoms felt like even among highly verbal children [7,19]. This was confirmed in our qualitative interviews, in which parents described the sorts of behaviors they use to identify when their child is in distress. Although these behaviors are non-specific and could indicate a host of underlying medical or psychiatric problems, they are useful as a signal that something is awry, and therefore deserve attention.

In some instances, parents shared that these GI symptoms made their children very aggressive or violent, to the point of having to call police or advise other siblings to stay away from the child with ASD. It's important to note that people with mental illness are much more likely to be victims of violence than to perpetuate[49]. The findings of this study, however, remind us that acts of aggression or violence may be indicators of severe pain or distress, and underlying medical issues need to be assessed as possible causes of these serious behaviors. Self-injurious behavior is also, unfortunately, common in ASD, and again, may point to distress due to a medical problem[50–52]. This should be considered in the context of emergency response and de-escalation of situations. Strategies for assessing and ameliorating symptoms may play an important role in crisis interventions.

The second major theme that emerged from the qualitative interviews was the impact that the child's GI symptoms have on their own functioning as well as the functioning and wellbeing of the family unit. A major focus of parents' discussion of this topic was around the challenges that GI symptoms posed for the child's ability to go to school and learn.

School challenges, and absenteeism in particular, have been associated with GI disorders and other medical conditions have been described in literature focused on typically developing children[53–58]. The high prevalence of GI symptoms in children with autism, with the evidence that these symptoms impair the quality of their education, begs for more research and more importantly, interventions that support children with GI issues by promoting accessibility. Qualitative interviews showed that social and extracurricular activities were also affected by a child's GI symptoms. Prior research demonstrates that children with ASD are less likely to participate in social, extracurricular or leisure activities, and that sensory, mental, behavioral, and motor issues influenced likelihood of participation[59,60]. Addressing GI-related challenges may encourage children with be more involved sand remove barriers to participation.

GI symptoms also had an important impact on the overall functioning and wellbeing of the family, and in particular, the ability of the family to leave the house, the family's stress level, and the family's financial wellness. Elsewhere in the literature, strong dietary aversions or special diets that are common in ASD have been shown to play a role in influencing the family's activities[61,62]. This study demonstrates that a child's toileting problems may also dictate the activities their families can pursue. This highlights not only the need for respite, but also for accessible locations for families who have a child with ASD.

Financial health can also be affected by GI issues. Aside from costs due to seeing medical providers, some families reported high costs of exploring complementary alternative medicines, including special diets and probiotics. This last finding stresses the importance of better understanding the safety and efficacy of complementary and alternative treatments, as over 70% of families with a child with ASD report using a CAM[63].

The last main theme of this qualitative work was the negative experiences that families with a child with ASD and comorbid GI symptoms often faced when seeking care. Some challenges were not necessarily unique to ASD, such as the long wait-times between receiving an appointment and seeing the physician, or dealing with financial stress and complex insurance systems. However, some obstacles were specific to ASD or greatly increased in this population, for example difficulties in bringing their child to a medical appointment. One parent in our study noted that their child was denied service immediately upon walking in because of his ASD symptoms.

Perhaps the most disturbing obstacle that parents faced when attempting to resolve their child's GI symptoms was the issue of not being taken seriously by medical professionals. This is not the first qualitative study of autism parents to share these sentiments. In a qualitative study of feeding challenges in children with ASD, a mother felt that her child's symptoms were viewed as behavioral problems due to autism and perhaps even dismissed by medical professionals for this reason. Another mother in that study who sought medical help for their child's GI issues also felt dismissed, explaining that her son's autism diagnosis meant that he was not regarded as a child with a physical illness.[46]

To our knowledge, this is the first qualitative study to explore family experiences with having a child with ASD with GI symptoms. Our study is limited, however, in that it examined GI symptoms in childhood, since it was in part designed to aid in the development of a GI questionnaire for children with ASD. Future work needs to include adults with ASD and inquire about GI and related symptoms across the life course. We also did not have as many individuals with ASD participate in the interviews, as we would have liked. Future analyses would benefit from a greater inclusion of individuals with ASD if possible.

In this qualitative study of children with ASD and GI symptoms, we summarized indicators of GI symptoms or pain, described how GI distress impacts the functioning of the child and the family, and demonstrated that families often have negative encounters with the medical community as they seek to alleviate their child's GI symptoms and pain. Findings from this qualitative study stress the importance of better understanding the landscape of GI-related issues in individuals with ASD, including the importance of accurate measurement of symptoms, the association with co-occurring mental and physical health issues, use of medical services as well as complementary and alternative medical treatments. GI symptoms in children with ASD place an incredible toll on the wellness of both the child and also the surrounding family.

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CHAPTER 3: GASTROINTESTINAL SYMPTOMS IN AUTISM SPECTRUM DISORDER: A REVIEW OF THE LITERATURE ON ASCERTAINMENT AND PREVALENCE

Coauthors: Carol Newill[1,2], Li-Ching Lee[3,2], Pankaj J. Pasricha[4,2], Dani Fallin[1,2]

[1]Department of Mental Health, Johns Hopkins Bloomberg School of Public Health; [2]Wendy Klag Center for Autism and Developmental Disabilities, Johns Hopkins Bloomberg School of Public Health; [3]Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health; [4]School of Medicine, Johns Hopkins University

3.1 Introduction

In his seminal paper first describing autism, Leo Kanner noted that 6 of the 10 children with autism "presented severe feeding difficulty from the beginning of life"[1]. Since then, many studies have explored the association between autism spectrum disorder (ASD), gastrointestinal (GI) symptoms, and diet[2–5]. The argument that individuals with ASD have a non-healthy gut stems from the apparent increased prevalence of GI symptoms, as cited widely in the literature since Kanner's 1943 paper[6–9]. For example, a study published in 2014 reported that 13% of their ASD sample had current frequent diarrhea, compared to 6.1% in the developmental delay group and 1.6% in the typically developing group[9]. Other symptoms were elevated in ASD as well, though they were not statistically significantly different across groups. Although five questionnaires (Autism Treatment Evaluation Checklist (ATEC) subscale, Autism Treatment Network (ATN) Gastrointestinal Symptom Inventory, Childhood Autism Risks from Genetics and Environment (CHARGE) Gastrointestinal History Questionnaire, Parental Concerns Questionnaire, and ATN Diagnoses and Problems form) that query GI symptoms have been designed for the ASD population, to our knowledge none have been formally assessed for validity[10]. Therefore, the true prevalence of various GI symptoms in this population is unknown.

Epidemiologists and experts in pediatric gastroenterology have recognized many limitations in the interpretation of published studies on the epidemiology of GI problems among children with ASD. In a report from a 2009 symposium of the North American Society of Pediatric Gastroenterology and its follow-up workshop, Coury et al. noted the very wide range of prevalence estimates of GI disorders in ASD, and recognized several methodological limitations among the studies[11]. These included retrospective study design and inappropriate control groups,

which potentially lead to measurement error and bias; enrollment of groups of children with ASD that are clinically heterogeneous, which generates wide estimates of symptom prevalence; bias in case selection, which may make the sample appear to have more or less symptoms than the underlying target population; as well as reliance on parent report of symptoms, which may provide incomplete or biased information, especially when symptoms are not defined precisely.

Buie et al. published a consensus report from a multidisciplinary panel on the evaluation and treatment of GI disorders and symptoms among people with ASD[10] noting the wide variability among prevalence estimates, and recommended the use of "validated instruments and outcome measures". The authors further noted that GI symptoms may contribute to problem behaviors among people with ASD, and that the problem behaviors may complicate the clinical recognition of GI disorders.

Ascertaining accurate estimates of GI symptoms in children is a challenging issue in general. For example, while the Rome II criteria for functional constipation required at least 2 weeks of a defined type of stool "for the majority of stools" or another type of stool "2 or more times per week"[12], the North American Society of Gastroenterology and Nutrition (NASPGHAN) defined constipation in terms of the timing of and the distress caused by defecation, present for at least 2 weeks[13]. Van den Berg et al. found that while some studies used these recognized, albeit disparate, criteria, other studies created their own criteria. They postulated that "it is unlikely that parents have comparable notions regarding constipation or about what constitutes normal bowel habits in children," and that both these sources of heterogeneity (the variety of definitions

and the variability of parental perceptions) contributed to the wide range of prevalence observed in their review of the literature[14].

However, these measurement challenges are compounded when assessing GI symptoms among children with social and communication difficulties, such as those with ASD. Even among neurotypical children, there can be low concordance between child and parent on intensity of pain/discomfort (children tend to rate pain/discomfort as more severe), pain during bowel movements, holding bowel movements, and heartburn, among others.[15] This low concordance may be more pronounced in younger age groups. Because studies of ASD usually rely on proxy respondents, it is likely that frequency estimates of GI symptoms and pain are biased in this population, and may be more biased among children in the younger age groups or with more severe autism. Other populations with neurodevelopmental disabilities such as cerebral palsy (CP) have experienced similar challenges in assessing the presence of GI symptoms. Early studies in CP used their own questionnaires and for children the questionnaires were filled out by parents[16,17]. A recent study among adults with CP used a combination of questionnaires in a two-step process[18], specifically a questionnaire based on Rome III criteria and, if the criteria for constipation were met, then the Patient Assessment of Constipation-Symptom Scale; this study had the advantage of asking adult participants for information about their own bowel habits. For this review, we focused on ASD, since this population has been a primary target of complementary alternative therapies that aim to heal the gut and prevent or treat ASD, as well as research attempting to prove vaccines cause ASD through a damaged gastrointestinal system. All of this research rests on having an accurate measure of gastrointestinal symptoms. The implications of not having a reliable and valid assessment tool for GI symptoms are especially

critical for the ASD population given the vulnerability of this population to potentially unsafe, ineffective interventions.

It is important to better understand the range of GI measurement approaches and their influence on symptom prevalence estimates in order to: a) accurately capture the prevalence of various GI symptoms, b) assess the safety and effectiveness of interventions on GI symptoms, and c) understand risk factors for and trajectories of ASD.

The aims of this study were to 1) describe the range of approaches to ascertaining GI symptoms and conditions in studies of ASD since 1980, 2) describe the range of estimates of prevalence across studies, and 3) assess how the variation in measurement approach is associated with GI symptom prevalence estimates. Lastly, we outline the critical components needed in a GI questionnaire, and hope that this review will provide insight that will help in the creation or modification of a reliable and valid tool for measuring GI symptoms among individuals with ASD.

3.2 Methods

We carried out a literature review; not a formal systematic review. However, we followed many of the best practices of PRISMA guidelines including explicit search criteria, inclusion/exclusion criteria, standardized extraction of the same fields, and resolution of extraction discrepancies across two reviewers. These are described in detail below.

3.2.1 Search Criteria

PubMed was used to find all studies published from 1/1/1980 to 1/31/2017, with a title including "Autism", "Autistic", "ASD", "Pervasive Development*", or "Asperger*", and with Medical Subject Headings terms "Gastrointestinal Disease" or "Signs and Symptoms, Digestive", or one of the following terms in the text: "gastrointestinal", "gastric", "gastritis", "gut", "GI", "intestine*". Reviews and articles not written in English were excluded. This search returned 386 studies. The asterisk (*) returns words that begin with the word truncated by the asterisk.

3.2.2 Exclusion Criteria, Information Extracted

From these 386 studies, exclusion criteria were: a) having fewer than 10 diagnosed "case" participants (ASD, PDD, etc.) (n=38); b) not ascertaining GI symptoms or diagnoses (n=66); c) animal studies (n=32); d) containing no data (n=3); e) including Andrew Wakefield as a coauthor (n=11); and f) review articles, hypothesis papers, meta-analyses, narratives, editorials and mathematical model papers (n=92). Andrew Wakefield et al.'s 1998 Lancet paper[19], which suggested the measles, mumps, rubella vaccine predisposes to behavioral regression, was retracted in 2010 due to incorrect elements of the paper as well as ethical violations. It was later discovered that Wakefield et al. had conducted fraud by picking data that agreed with their hypothesis[20]. The claims made in the original paper continue to have lasting effects on vaccination rates[21]. For these reasons, we chose to omit studies on which he was a coauthor. As shown in Figure 1, 144 studies remained [Supplementary Table 1].

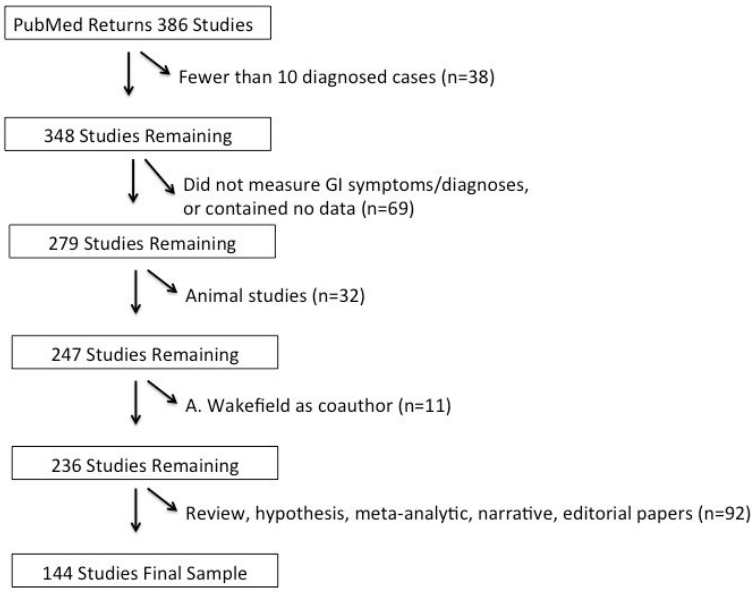

Figure 1. Exclusion Flowchart, from Initial 386 Studies to Final Sample of 144 Studies.

Figure 1 depicts the exclusion flow chart, beginning with 386 studies that were returned from PubMed. After applying our exclusion criteria, 144 studies remained in the final sample of studies we reviewed.

Information was extracted from each of 144 studies by one of two authors (CH & CN). Both authors examined a subset of the studies, and questions regarding any study were resolved together. Information extracted included study design, demographic information, ASD diagnostic criteria, and characteristics of data-collecting methods.

3.2.3 Prevalence Estimate Subset

Of the 144 studies, a subset was identified that contained prevalence estimates, in order to summarize GI symptom prevalence estimates across studies. Studies were excluded if: entry into the study, or the sampling frame, was based on the presence or absence of GI symptoms/disorders (including GI-related medical comorbidities) (n=20); the study included/excluded participants based on diet (n=2); the study was experimental (n=26); or the

study did not report GI symptom/diagnosis estimates (n=12). The flowchart shows these exclusions (Figure 2).

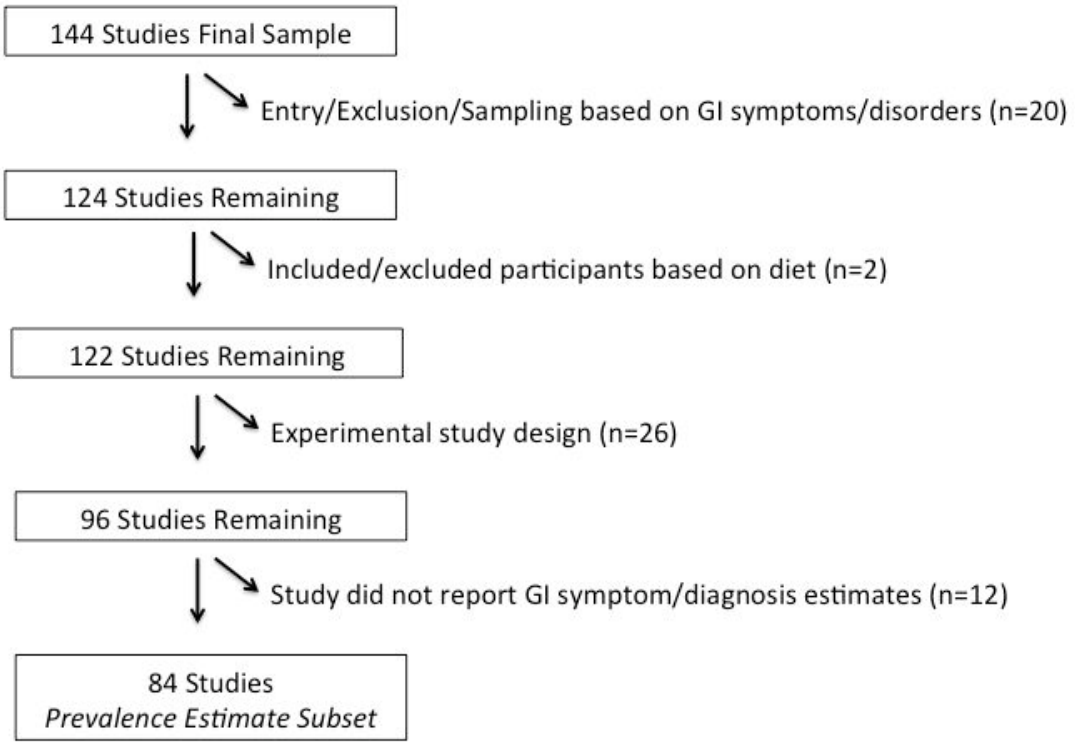

Figure 2. Exclusion Flowchart, Resulting in the Prevalence Estimate Subset of 84 Studies.

Figure 2 depicts the exclusion flow chart, beginning with the 144 studies that we reviewed. After applying our exclusion criteria, 84 studies remained in the final sample of studies from which we summarized the prevalence estimates of GI symptoms.

From the 84 studies that qualified to be included in the prevalence estimate subset, we extracted proportions of various GI symptoms and noted whether GI symptoms were assessed at more than

one time point in the study. We summarized the proportions across studies for diarrhea, constipation, abdominal pain/discomfort, nausea/vomiting, bloating/flatulence, reflux, soiling/incontinence, and difficulty/pain while stooling. We also summarized non-specific (did not refer to a specific symptom) or aggregate (referred to more than one symptom) variables, such as "gut complaints", "any symptom", or "had either diarrhea or constipation". For studies in which there were subgroups, such as children with/without language regression, or when there was more than one time point, we took the average across these subgroups. Studies that used scores or mean number of symptoms, rather than percentages, were excluded when calculating the summary prevalence measure.

We assessed relationships between GI symptom proportions and various study characteristics using ANOVA tests. We estimated the association between the mean proportion for each GI symptom and each study characteristics of interest (age, geographic region, publication year, diagnostic category, primary goal of study, type of study sample, study design, who reported the symptoms, and type of questionnaire). P-values less than 0.05 were considered statistically significant. All statistical analyses were performed using RStudio Version 0.98.1091[2223].

3.3 Results

3.3.1 Characteristics of Studies

Study characteristics are summarized in Table 1. The number of study participants ranged from 10 to 4927 individuals (median 73). The source of study participants was clinic-based in 57 studies, population-based in 18 studies, both clinic- and population-based in 1 study, enriched-

risk in 2 studies, and in 66 studies, insufficient information was provided to determine the source population.

There were 62 case-control studies, 48 cross-sectional studies, (15 of which included a comparison group), 25 experimental studies, 6 cohort studies, and 3 with mixed study designs. One hundred and sixteen studies were observational in nature, 25 were experimental, and 3 had both observational and experimental components.

The primary goals of the studies were to: 1) assess the relationships of GI symptoms and conditions with any other variable (n=51); 2) compare GI symptom estimates among groups of participants (e.g. ASD, Developmental Delay, PDD-NOS) (n=24); 3) assess outcomes of experimental treatments (n=24); and 4) other primary goal (n=42). Three studies had both primary goals of comparing prevalence estimates as well assessing associations between GI symptoms/conditions and other variables.

Table 1. Characteristics of Studies and Demographic Information (count (%) or median (range))

	N=144 studies
Sample Size	73 (10-4927)
Type of study sample	
Clinic-based	57 (40%)
Population-based	18 (12.5%)
Clinic-based & Population-based	1 (0.7%)
Enriched-risk	2 (1.4%)
Other, or insufficient description	66 (46.0%)
Study Design	
Experimental	25 (17.4%)
Cross-sectional	33 (22.9%)
Cross-sectional with comparison group	15 (10.4%)
Case-control	62 (43.1%)
Cohort	6 (4.2%)
Mixed designs	3 (2.1%)
Primary goal of study	
Obtaining estimate of GI symptoms/comparing across Outcomes	24 (16.7%)
Associating GI symptoms with other variable	51 (35.4%)
Obtaining estimate of GI symptoms/comparing across outcomes and Associating GI symptoms with other variable	3 (2.1%)
Intervention effect on outcomes	24 (16.7%)
Other	42 (29.2%)
Region of Sample	
Africa	2 (1.4%)
Asia	10 (6.9%)
Europe	22 (15.3%)
Oceania	8 (5.6%)
South America	1 (0.7%)
United Kingdom	12 (8.3%)
US/Canada	89 (61.8%)
Race/Ethnicity (among US/Canada, UK studies)	
All white, majority white	46 (45.5%)
Mostly Latino/Hispanic	3 (3.0%)
Not specified	49 (48.5%)
No distributions provided	3 (3.0%)
Age categories, median of means (years)	
<2	0 (0%)
2-5	9 (10.3%)
5-12	68 (78.2%)

13-18	5 (5.7%)
18+	5 (5.7%)
Missing	57 (39.6%)
Diagnostic Categories	
DSM III-R[a]	1 (0.01%)
DSM IV[a]	73 (50.7%)
DSM V[a]	6 (4.2%)
ICD-9 (but no DSM code)	5 (3.5%)
ICD-10 (but no DSM code)	11 (7.6%)
ICD-9 and ICD-10, but no DSM code	1 (0.01%)
No DSM or ICD code reported, but used ADI-R and/or ADOS	23 (16.0%)
No DSM or ICD code, or ADI-R and/or ADOS, but used Medical Record	1 (0.01%)
Other, Not Specified	23 (16.0%)

[a]These studies may or may not have included ICD codes as well.

3.3.2 Demographic Information

Of the 144 studies, 61.8% were conducted in the United States or Canada, 15.3% in Europe, 6.9% in Asia, with a smaller proportion in the United Kingdom (8.3%), Oceania (5.6%), Africa (1.4%), and South America (0.7%) (Table 1). Of the 101 studies in the US, Canada, or UK, over half (51.5%) did not report the race/ethnicity composition of their study sample. Among the 49 studies that did report race/ethnicity, 94.0% of them included exclusively white participants or a very small proportion of participants were non-white. The ages of participants ranged from <1 to 64 years old. The median age within studies ranged from 4.5 to 36 years, and the median age (median of medians) across studies was 6.7 years (Table 1). Of the 87 studies that reported age, nine (10.3%) had a median of means between the ages of 2-5 years, 68 studies (78.2%) between ages 6-12 years, 5 studies (5.7%) between the ages of 13-18 years, and 5 studies (5.7%) ages 18 or older.

3.3.3 Diagnostic Classification of ASD Individuals

Studies used a variety of methods to diagnose ASD. Most studies either relied on prior assessment of ASD by a practitioner (sometimes with written verification) or assessment with the ADI-R, ADOS, or Mullen. Some studies relied on parent interviews as well as observation by a clinical team involving psychologists, psychiatrists, and/or developmental pediatricians.

A variety of diagnostic classifications were used among studies. Most studies required that participants meet DSM-IV criteria for Autistic Disorder, with or without including Asperger's Disorder or PDD-NOS (n=73, 50.7%), or DSM-V criteria for Autism Spectrum Disorder (n=6, 4.2%), and one earlier study used DSM-III-R (0.01%). These studies may or may not have used

an ICD code in addition to DSM codes. Sixteen studies (11.1%) used an ICD code but did not use a DSM code. In addition, twenty-three (16.0%) studies did not report the specific DSM or ICD diagnosis but did use the ADI-R or ADOS. Twenty-three studies (16.0%) did not use ADI-R or ADOS and did not report a DSM or ICD diagnosis (Table 1). One study relied on medical records for diagnosis without specifying DSM or ICD category (0.7%).

3.3.4 Methods of Ascertainment of GI Symptoms and Conditions

Studies used the following methods to assess GI symptoms or diagnoses: 83 of 144 studies used questionnaires administered exclusively to parents or caregivers; 3 used questionnaires administered to either parents/caregivers or teachers; 6 used questionnaires administered to parents/caregivers or to the individuals with ASD themselves (Table 2). Only 18 studies used both questionnaires administered to parents/caregivers and also information from a professional medical source (8 from physicians and 10 from medical records). Of the 8 studies that involved a physician in the ascertainment of GI symptoms, only one mentioned that the physician was a gastroenterology specialist[24]. Medical record studies may have depended on GI specialists, but often who diagnosed the medical condition was not specified. Of the 8 studies that involved a physician, 3 mentioned that some participants also underwent endoscopic procedures. Two of these studies described that a portion of participants had undergone endoscopy and/or colonoscopy in their initial GI evaluation, independent of the study. In one study[25], about 17% of individuals underwent endoscopic procedures (half of which showed pathologic results), and in the other study[26] 15% underwent both an endoscopy and colonoscopy as well as a biopsy of GI mucosa. The third study[24], also the one that incorporated a GI specialist, stated that endoscopic procedures were performed when clinically relevant, as part of the study. Thirty-

give percent (35%) of children with ASD and GI dysfunction underwent an esophagogastroduodenoscopy, 7.5% received a flexiblesigmoidoscopy, and 10% received a colonoscopy.

Fifteen studies used medical records exclusively. One study used claims data. Eighteen studies (12.5%) did not clearly specify who reported the GI symptoms or diagnoses (Table 2); 9 of these 18 studies used a questionnaire, although who filled out the questionnaire was not clear.

Of the 119 studies that used a questionnaire, 68 created their own instrument (i.e. a questionnaire never used before in other studies), while 51 studies used or modified an existing instrument. Three of the 68 studies that used their own approach noted that their method was informed by the literature, in particular the consensus report on GI disorders in people with ASD by Buie et al[10]. The questionnaires that were used the most often were the Rome II (n=4) or III criteria (n=12), The Gastrointestinal Severity Index (Schneider)[27] (n=5), the Autism Treatment Network Gastrointestinal Symptom Inventory[6] (n=3) and the Bowel Symptom Questionnaire (Smith)[28] (n=3) (Table 2). Symptom diaries (concurrent daily or weekly observations) were used in 9 studies. Supplementary Table 2 lists which studies used which questionnaires.

Table 2. Measurement Approaches Across Studies (count)

	N=144 Studies[b]	N=84 Studies[bc]
Respondent of GI Symptoms/Diagnoses		
Parent/caregiver questionnaires only	83	52
Medical Records	15	13
Parent/caregiver and medical records	10	7
Parent/caregiver and physician	8	6
Parent/caregiver or self	6	0
Parent/caregiver or teacher	3	0
Claims data	1	1
Respondent unclear, not specified	18	5
Measurement Approach		
Daily or weekly symptom diaries	9	0
Medical Records	16	13
Questionnaire	119	68
Questionnaire		
Custom design for study	65	44
Custom design for study, informed by literature	3	2
Based on Existing Instrument	51	22
Rome III	12	3
The Gastrointestinal Severity Index (Schneider)	5	2
Rome II	4	3
Autism Treatment Network (ATN)- The Gastrointestinal Symptom Inventory	3	3

Questionnaire (continued)	N=144 Studies[b]	N=84 Studies[bc]
Bowel Symptom Questionnaire (Smith)	3	2
Childhood Autism Risks from Genetics and Environment (CHARGE)		
Gastrointestinal History Questionnaire (GIH)	2	2
GI Symptom Questionnaire (Chandler)	2	2
Global Behavior Rating Scale	2	0
Secretin Outcome Survey	2	0
Child Behavior Checklist (CBCL)	1	1
Gastrointestinal Symptom Rating Scale (GSRS) (Chisholm)	2	1
Lower Urinary Tract Symptoms (LUTS)	1	1
Modified GI symptom severity index questionnaire	1	1
Parental Concerns Questionnaire & ATN Diagnoses and Problems & Clinician Form, & Health and Mental Health History	1	1
ATEC subscale	1	0
Autism Treatment Evaluation Checklist (ATEC) subscale & Global Impressions Survey	1	0
Global Behavior Rating Scale & Additional Rating Scale	1	0
Global Impressions Survey	1	0
Irritable Bowel Syndrome (IBS) Global Improvement Scale	1	0
National Health Interview Survey (NHIS) questionnaire	1	0
Rome III & their own approach	1	0
Safety Monitoring Uniform Report Form	1	0
Safety Monitoring Uniform Report Form & Global Impressions Survey	1	0
Side Effects Review Form	1	0

[b]Categories are not mutually exclusive. [c]Eighty-four studies from the total set of 144 studies were used to summarize prevalence estimates of GI symptoms across the studies.

3.3.5 GI Symptoms and Conditions Ascertained in the Studies

The following symptoms and/or diagnoses were measured in the 144 studies (Table 3): constipation/chronic constipation (n=94), diarrhea/chronic diarrhea (n=95), abdominal pain or discomfort (n=66), nausea or vomiting (n=53), stool qualities or patterns (frequency, color, smell, presence of mucus) (n=51), bloating, gas, or flatulence (n=47), reflux or heartburn (n=41), food selectivity issues or allergies (n=36), soiling, incontinence or bedwetting (n=25), pain or difficulty having a bowel movement (n=20), and colic (n=4). Seventy-five studies measured a non-specific GI symptom or a variable that was a combination of several symptoms, such as constipation, diarrhea, or abdominal bloating. Twenty-three of those seventy-five studies reported only the category of non-specific/aggregate symptoms, while the other 52 reported specific symptoms as well. The variables that were analyzed most commonly for an association with GI symptoms/conditions were the diagnostic outcome group of the study (e.g. ASD/Developmental Delay/PDD-NOS) (n=46), behavioral, psychological, and IQ variables (n=28), and demographic variables (n=13) (Supplementary Table 3).

Table 3. Symptoms Ascertained Across 144 Studies (count (%))

Symptom[b]	N=144 studies
Constipation	89 (61.8%)
Chronic/Persistent Constipation	5 (3.5%)
Diarrhea	86 (59.7%)
Chronic/Persistent Diarrhea	9 (6.3%)
Abdominal pain or discomfort	66 (45.8%)
Nausea or vomiting	53 (36.8%)
Stool qualities or patterns	51 (35.4%)
Bloating, gas, or flatulence	47 (32.6%)
Reflux or heartburn	41 (28.5%)
Food sensitivities, eating issues	36 (25.0%)
Incontinence or bedwetting	25 (17.4%)
Pain or difficulty having bowel movement	20 (13.9%)
Colic	4 (2.8%)
Non-specific GI symptom/Aggregate of symptoms with or without specific symptoms	75 (52.1%)
Only non-specific GI symptom/Aggregate of symptoms	23 (16.0%)

[b]Categories are not mutually exclusive.

3.3.6 Prevalence Estimate Subset

The distribution of measurement approaches in the subset of 84 studies is summarized in Table 2. Sixty-eight of the 84 studies used questionnaires, 13 used medical records, and none used symptom diaries.

The symptoms with the highest median prevalence proportion across studies were "any GI symptom/aggregate of symptoms" (46.8%), constipation (22.0%), chronic/persistent constipation (19.7%), diarrhea (13.0%), chronic/persistent diarrhea (16.2%), and abdominal pain or discomfort (14.0%) (Table 4). The ranges of prevalence proportions were quite wide. Among the 62 studies that reported results on a category of "any" GI symptom, the range was 4.2 to 96.8% of participants. For constipation (n=32), the range was 4.3 to 45.5%, and for diarrhea (n=29), the range was 2.3 to 75.6%.

A number of symptom prevalence proportions varied significantly by study characteristics. There were multiple differences in symptom proportions depending on who reported the symptoms (Figure 3). Proportions of diarrhea, abdominal pain/discomfort, nausea/vomiting, and bloating/flatulence/gas, all different significantly by respondent type ($p<0.05$; Figure 3). Symptoms had the highest prevalence proportion in studies that did not specify the respondent of symptoms, with the exception of the reflux symptoms, which did not have studies in this category. Reflux symptoms were highest in studies that used medical records or claims data.

Table 4. Prevalence Proportions of GI Symptoms/Conditions Among 84 Studies (median (range))

Symptom	Estimate	Number of Studies Contributing to this Estimate; Reported Measuring Symptoms
Any GI symptom/Aggregate of symptoms	46.8 (4.2, 96.8)	62; 64
Constipation	22.0 (4.3, 45.5)	32; 53
Chronic/Persistent Constipation	19.7 (8.8, 38.5)	6; 6
Diarrhea	13 (2.3, 75.6)	29; 51
Chronic/Persistent Diarrhea	16.2 (7.1, 37.0)	9; 9
Abdominal Pain/Discomfort	14 (2.1, 46.6)	20; 35
Nausea or Vomiting	6.1 (1.3, 21.5)	13; 24
Stool qualities (frequency, color, small, mucus)	--	--
Bloating, Flatulence, Gas	12.5 (0, 55.2)	14; 24
Reflux, heartburn, acidic stomach, GERD, spitting up	7.4 (0, 21.5)	11; 21
Food selectivity/sensitivities, allergies	--	--
Soiling, incontinence, bedwetting	12.5 (2.3, 24.0)	5; 12
Difficult with bowel movements, pain/straining while stooling	6.2 (6.2, 6.2)	1; 10

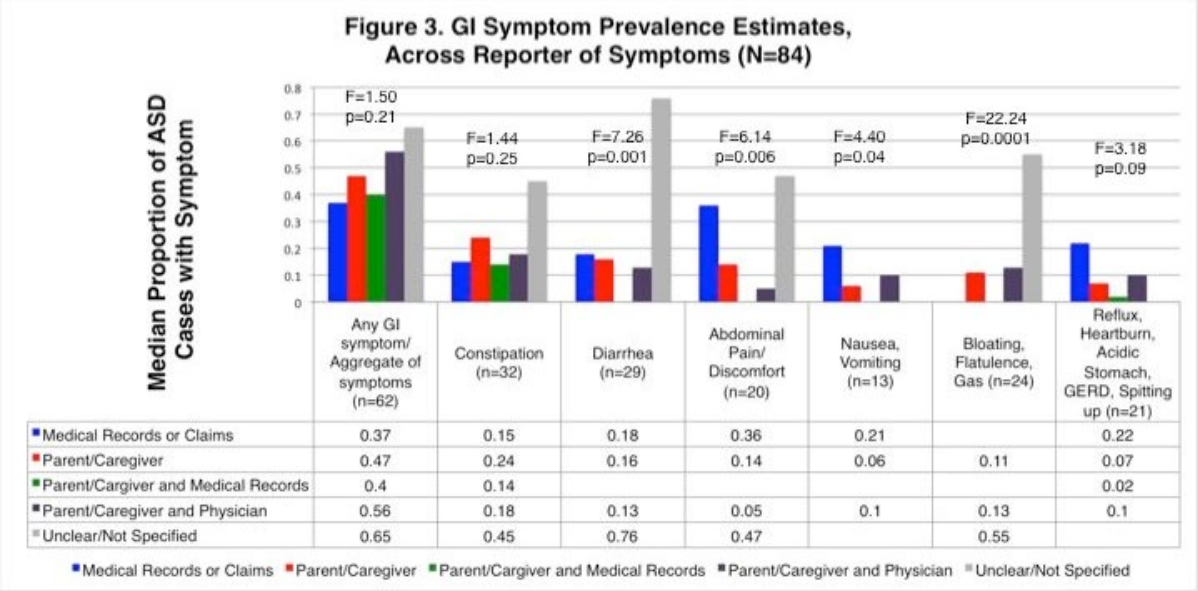

Figure 3. GI Symptom Prevalence Estimates, Across Reporter of Symptoms (N=84).

Figure 3 summarizes the associations between specific GI symptoms and the respondent of GI symptoms in 84 studies. GI symptom proportions are the median prevalence proportions across 84 possible studies. The number of studies contributing to the estimate is shown in the horizontal axis. An ANOVA test was carried out for each symptom to test for differences in estimates across types of respondent. P-values are indicated below the F-values.

Notably, both abdominal pain/discomfort and reflux proportions differed across age groups, with the highest proportions for both symptoms being in studies in which the mean age range was 13-18 ($p<0.1$, $p<0.005$, respectively, data not shown). Soiling/incontinence proportions different by the primary goal of the study; studies with the primary goal of comparing GI symptom proportions across outcome groups had the highest proportions ($p<0.05$, data not shown). Chronic or persistent constipation proportions differed significantly across study design types, with the highest proportions in observational studies with a comparison group ($p<0.05$, data not shown). Prevalence proportions of constipation differed significantly across types of study

samples, with clinic-based and enriched-risk samples having the highest proportions ($p<0.03$, Table S3).

No symptom prevalence proportions differed significantly ($p<0.05$) or had a discernable association with diagnostic category, geographic region, publication year, or by the type of questionnaire (e.g. ATN, Rome criteria, Child Behavior Checklist). We were unable to examine associations with other variables such as intellectual disability or verbal ability due to the scarcity of proportions specific to these subgroups.

3.4 Discussion and Conclusion

3.4.1 Key Findings

In this review of 144 studies published 1980-2017, a broad range of approaches to ascertaining the presence of GI symptoms and conditions was observed. Most studies relied on questionnaires given to parents or caregivers, some used medical records, and a few used concurrent observations such as symptom and stool diaries or results of clinical diagnostic examinations. Of the 84 studies used in the calculation of GI prevalence estimates, only one obtained information directly from the participants and none used observational symptom diaries. Some studies were based on population samples while others recruited participants from clinical settings. Among the 116 observational studies, 82 studies (71%) included a comparison group. Future studies measuring GI symptoms in ASD should continue to use a comparison group in order to determine the relative risk magnitude of various GI symptoms.

Importantly, we found that over half of the studies that used a questionnaire used their own approach, while the rest used an instrument that had been previously developed. Among the existing instruments, only five (ATEC subscale, ATN Gastrointestinal Symptom Inventory, CHARGE Gastrointestinal History Questionnaire, Parental Concerns Questionnaire, and ATN Diagnoses and Problems form) were designed with individuals with ASD in mind, and none have been formally validated. This diversity in measurement approaches makes it difficult to derive an accurate epidemiologic estimate of GI symptoms and an understanding of the risk factors that contribute to ASD and GI symptoms. The symptoms that were most commonly ascertained across the studies reviewed here were constipation, diarrhea, abdominal pain or discomfort, and nausea or vomiting. Twenty-three (16.0%) of the studies only reported a non-specific or aggregate GI variable, making it difficult to specify the particular symptoms that are reported in this population.

Not surprisingly, a wide range of proportions of symptom prevalences was observed. Notably, the proportion of non-specific/aggregate GI symptoms ranged from 4.2 to 96.8% of participants. Yet, more specific symptoms also had a wide range of proportions. For example, diarrhea, which is arguably one of the easier symptoms to detect or measure, still had a range of 2.3 to 75.6% across studies. It is not clear if these results are specific to ASD or reflect a broader group of central nervous system disorders. Some research suggests that individuals with ASD may experience a higher prevalence of GI symptoms than other developmental delay groups, however, the lack of precision and validity around measuring GI symptoms makes it difficult to confirm this[9]. Importantly, current data may represent an under-estimate of GI symptoms and

disorders, because behaviors that are reflective of GI distress (aggression, disruptive behaviors, self-injury) may be interpreted as features of ASD and therefore may go uninvestigated.

Ascertaining the presence of even one of the more common GI symptoms, functional constipation (that is, constipation not due to anatomic structural disorders), is a challenging problem, even among children who do not have developmental disorders. As noted in a 2006 review by van den Berg et al., the need to rely on reporting by parents, the lack of standardization of the definition of specific symptoms, as well as the reliance by physicians on parental interpretation, all contribute to the problem of inaccurate symptom measurement[14].

In support of this, we found that many GI symptom prevalence estimates were significantly associated with study characteristics. Diarrhea, abdominal pain/discomfort, nausea/vomiting, and bloating/flatulence/gas differed significantly by respondent type, soiling/incontinence differed by the primary goal of the study, and constipation differed significantly across study design types as well as type of study sample.

Our review has important limitations. First, we used a single database (PubMed), so studies that measured GI symptoms in ASD, but were published in journals not available through PubMed, are not reflected in this review. Secondly, since many studies did not specify the prevalence of GI symptoms in subgroups of their populations, such as individuals with an intellectual disability or who are non-verbal, we were unable to determine how GI symptoms differed across these groups. Despite these limitations, this review, and previous ones, highlight the need for standardized, validated tools to assess GI symptoms among people with ASD. There are a few

notable examples of questionnaires that do assess GI symptoms and have been designed for ASD, yet each has current drawbacks, and none of the GI-specific sections have been validated. We briefly summarize three of these questionnaires and describe their strengths and limitations.

3.4.2 Strengths and Limitations of Current GI Symptom Questionnaires for ASD populations

The Autism Treatment Evaluation Checklist (ATEC) subscale[29], developed by Bernard Rimland and Stephan M. Edelson at the Autism Research Institute, is a one-page form to be filled out by parents, teachers, or caregivers. The four subsections of the scale are 1) Speech/Language Communication, 2) Sociability, 3) Sensory/ Cognitive Awareness, and 4) Health/Physical/Behavior. The ATEC provides subscale scores and a total score, used to monitor how the child is doing over time, or following an intervention. The Health/Physical/Behavior section consists of 25 items, which the individual rates as Not a problem; Minor Problem; Moderate Problem; Serious Problem. The items that have to do with the gastrointestinal system are: bed-wetting; wets pants/diapers; soils pants/diapers; diarrhea; constipation; eats too much/too little; extremely limited diet[29,30]. The strengths of the ATEC subscale are that it covers multiple constructs of ASD through its four subsections, it allows for a severity rating, it includes items on GI-related issues such as diet, bed-wetting and incontinence, and it has been used in a number of studies. Further, the ATEC total and sub-scale scores, including the health/physical/behavior subscale, have demonstrated good reliability and validity [31,32]. However, diarrhea, constipation, and incontinence/bed-wetting make up only 5 of the 25 items in this subscale, limiting its usefulness for assessing the breadth of GI symptoms and distress in this population. Further, no definitions of symptoms are provided.

The Childhood Autism Risks from Genetics and Environment (CHARGE) Gastrointestinal History Questionnaire is a parent-administered questionnaire. The questionnaire includes 10 Likert scale items on current gastrointestinal symptoms, food allergies, and dietary restrictions (0=never; 1=rarely; 2=sometimes; 3=frequently; 4=always). The symptoms assessed are abdominal pain, gaseousness/bloating, diarrhea, constipation, pain on stooling, vomiting, sensitivity to foods, difficulty swallowing, blood in stool, and blood in vomit, as well as four yes/no questions about presence of food allergies, diet restrictions, food dislikes, and whether the child has ever received a GI diagnosis. In addition, the form includes open-ended questions for parents to list food allergies, reasons for diet or food restrictions, and what GI condition(s) have been diagnosed[9,33]. The strengths of this questionnaire are that it assesses many GI symptoms, provides information on frequency, has many questions on mealtime behaviors and diet, and allows parents/caregivers to provide qualitative data. The major limitation of this questionnaire is that it doesn't incorporate behavioral symptoms that may reflect GI distress.

The Autism Treatment Network Gastrointestinal Symptom Inventory is an especially useful questionnaire[34]. It is comprised of 35 items, plus additional items if a participant exhibits certain symptomatology, totaling 77 items. Parents fill out the inventory, which includes questions about presence, duration, and nature of a number of GI symptoms. For each item, parents are asked "has your child experienced any of the following gastrointestinal (tummy) symptoms?", to which they can reply with "Yes", "No", or "Unsure". For symptoms that are present, parents can also specify the duration of the symptoms (<3 months, 3-5 months, 6-11 months, 1 year or longer). This inventory is scored to provide binary variables for individual symptoms, any GI symptoms, and can provide the total number of GI symptoms experienced. In addition, the

inventory allows for branching into specific types of symptoms (abdominal pain, abnormal bowel movements, reflux, and food insensitivity), which allows for the estimation of these symptom categories. Unfortunately, the tool has not been validated [6,35,36]. The strengths of this questionnaire are that it provides information on duration of symptoms, assesses a number of GI symptoms, and queries behaviors that might be reflective of GI distress. The limitation of this questionnaire is that mealtime behaviors or dietary practices are not explored to any great degree despite their potential role in the expression of GI symptoms.

3.4.3 Recommendations for Creation and Psychometric Testing of GI Questionnaire

To our knowledge, this is the first literature review to assess GI symptoms in studies of ASD reaching back to 1980 and to examine study design features, particularly approaches to measurement of GI symptoms, for associations with GI symptom prevalence estimates. This study builds on McElhanon et al.'s meta-analysis of 15 studies which found that studies relied primarily on parental reports or chart reviews, and used a wide variety of definitions of GI symptoms[37]. They too recommended the development of "a standardized measure focusing on GI issues among children with ASD" and the use of a toileting diary of visual observations. Our review also confirms Dalton et al.'s hypothesis that the wide variability in prevalence estimates in ASD may be due to factors including the definition and type of GI symptoms, the sample of children, and the method by which the symptoms are investigated[38].

A standardized method for the ascertainment of GI symptoms and conditions must be designed for people with ASD, in order not only to have reliable and valid prevalence estimates but also to compare GI symptom estimates with various risk factors, and to evaluate the outcome of trials or

interventions. We recommend that an expert panel of clinicians, researchers, and individuals with ASD or their families be consulted to construct an item pool of symptoms and behaviors that are prevalent, meaningful, and relevant to the ASD population. The questionnaire should include not only GI symptoms but also stool patterns and qualities, mealtime and dietary behaviors, and behaviors that might be indicative of GI distress. In individuals who have difficulties communicating, behaviors or co-morbid conditions such as self-injury, aggression, chewing on non-edibles, putting pressure on the abdomen, sleep disruption or fragmentation, and any other unusual behaviors may be an indication of GI distress[10]. We refer the reader to the Consensus Report by Buie et al. for more vocal and motor behaviors and changes to overall state that may be indicative of abdominal pain or discomfort in people with ASD[10]. Not querying these behaviors could lead to an individual with ASD being incorrectly classified as not having a GI disorder or being in pain. This is relevant in research as well as clinical settings, where a patient's report of symptoms or pain typically precedes seeking care. Assessing for behaviors that might reflect GI distress as part of the clinical diagnostic process may help identify individuals in need of treatment. Although these behaviors and co-morbid conditions alone may not allow a questionnaire to be able to correctly diagnose a specific GI disorder such as reflux, Crohn's disease, ulcers, or tight rectal sphincter, it may indicate the need for a more comprehensive evaluation including endoscopy or colonoscopy when clinically indicated. Questions about GI symptoms should also include definitions, and information about duration, frequency, and severity. If possible, direct observations and prospective diaries of stool patterns, symptoms, and behaviors should be incorporated. After constructing the item pool, the GI questionnaire should be tested in a pilot sample of parent/caregiver/self-reporters for reliability and validity. We recommend factor analysis also be carried out to determine how individual

items map onto constructs. These results, as well as feedback from participants, should be used to revise the questionnaire before administering to a second, independent sample of individuals. Although the types of reliability and validity that can be assessed will vary by study, we suggest that internal consistency, test-retest reliability, content validity, criterion-validity, construct validity, and exploratory and confirmatory factor analyses are important analyses to consider when psychometrically testing this questionnaire. Importantly, no GI questionnaire based on parent/caregiver/self-report data will be able to diagnose specific disorders. However, A GI questionnaire represents a rapid, feasible way to assess symptoms, and could be combined with physician evaluation and diagnostic tests when relevant to a specific condition of interest in particular settings.

In the absence of a standardized approach to measuring GI symptoms, current reports should at the minimum state explicitly which questionnaire was used, who filled out the questionnaire, the ages of participants, study design, and other pertinent characteristics. We found that 12.5% of studies did not specify who reported symptoms, and 46% of studies did not describe their study sample with enough detail to know if it was a clinic-based sample, population-based sample, or enriched-risk sample. This is especially problematic given that studies which did not specify who reported the GI symptoms tended to have significantly higher proportions of diarrhea, abdominal pain/discomfort, bloating, as well as non-specific GI complaints.

Another troubling finding is that among studies in the US, Canada, and UK, 48% did not specify the race/ethnicity distributions of their sample, and of those that did, 94% were comprised of all or almost all white individuals. This highlights the dearth of racial or ethnic diversity among

participants in ASD research studies and begs for a greater focus on underrepresented populations. Similarly, the vast majority of studies did not report any information on socioeconomic status, making it difficult to assess how this impacts a family's ability to access GI specialists for diagnosis and treatment. This also reinforces the need for a more diverse sampling of individuals, greater reporting of socioeconomic variables, and an examination of how care is influenced by factors such as race, education, and income.

In summary, we found that ASD studies used a broad array of approaches to measuring the presence of GI symptoms and conditions, that most replied on parent or caregiver reports, and that the prevalence of GI symptom estimates across studies was very wide. Further, GI symptoms estimates were significantly associated with study characteristics such as respondent type, goal of the study, study design, type of study sample, and age of the participants. The current state of measuring GI symptoms in ASD hinders our ability to judge how symptoms vary over time and with other factors such as diet, sensory aversions, medication, psychological factors including anxiety or depression, the development of the child, and the various interventions that are explored. This review highlights the lack of consensus regarding frequency and subgrouping of GI symptoms in ASD. We argue that ASD individuals, their families, and the research community in general, would benefit from a standardized and valid approach to assessing GI symptoms in ASD. Psychometric testing of a GI questionnaire is an important first step in establishing the epidemiology of GI symptoms in individuals with ASD. If a reliable and valid open-access questionnaire were available to researchers, GI estimates across studies would be more comparable. By designing a questionnaire that has an item pool informed by an expert panel, that includes behaviors that could be indicative of GI distress

(aggression, self-injury, pushing abdomen, etc.), and that undergoes psychometric testing and revision, we think GI estimates will be more precise, accurate, and the items queried will be of interest to researchers, clinicians, and individuals or families with ASD. The consistent and accurate measurement of specific symptoms is crucial to understanding the role that the gut plays in the expression and course of ASD.

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Table S2. Map of which studies used which GI questionnaires.

Questionnaires Used in Studies
ATEC subscale
Ghosh et al. / 2015 / Journal of Clinical and Diagnostic Research
ATEC subscale and Global Impressions Survey
J B Adams et al. / 2011 / BMC Pediatrics
ATN The Gastrointestinal Symptom Inventory
Mazurek et al. / 2013 / Journal of Abnormal Child Psychology
Abdelrahman et al. / 2015 / Research in Developmental Disabilities
Mazefsky, Schreiber, Olino, & Minshew / 2014 / Autism : The International Journal of Research and Practice
Bowel Symptom Questionnaire
Wang et al. / 2012 / Digestive Diseases and Sciences
Wang et al. / 2013 / Molecular Autism
Wang et al. / 2011 / Applied and Environmental Microbiology
CHARGE GIH questionnaire
Chaidez, Hansen, & Hertz-Picciotto / 2014 / Journal of Autism and Developmental Disorders
Hansen et al. / 2008 / Ambulatory Pediatrics
Child Behavior Checklist
Fulceri et al. / 2016 / Digestive and Liver Disease
GI symptom questionnaire
N. Dalton et al. / 2014 / Autism Research
N. R. Dalton et al. / 2016 / Autism Research
Global Behavior Rating Scale
Saad et al. / 2015 / Clinical Psychopharmacology and Neuroscience : The Official Scientific Journal of the Korean College of Neuropsychopharmacology
Levy et al. / 2003 / Archives of Disease in Childhood
Global Behavior Rating Scale and Additional Rating Scale
Munasinghe, Oliff, Finn, & Wray / 2010 / Journal of Autism and Developmental Disorders
Global Impressions Survey
James B Adams & Holloway / 2004 / Journal of Alternative and Complementary Medicine (New York, N.Y.)
GSRS Chisholm

D.-W. Kang et al. / 2017 / Microbiome

Health and Mental Health History

Greenlee, Mosley, Shui, Veenstra-VanderWeele, & Gotham / 2016 / Pediatrics

IBS global improvement scale

Handen et al. / 2009 / Journal of Autism and Developmental Disorders

LUTS

Gontard, Pirrung, Niemczyk, & Equit / 2015 / Journal of Pediatric Urology

Modified GI symptom severity index questionnaire

Pusponegoro, Ismael, Sastroasmoro, Firmansyah, & Vandenplas / 2015 / Pediatric Gastroenterology, Hepatology & Nutrition

NHIS questionnaire

Phillips et al. / 2014 / Maternal and Child Health Journal

Parental Concerns Questionnaire & ATN Diagnoses and Problems–Clinician form

Greenlee et al. / 2016 / Pediatrics

Rome II

Pang & Croaker / 2011 / Pediatric Surgery International

M. Valicenti-McDermott et al. / 2006 / Journal of Developmental and Behavioral Pediatrics : JDBP

M. D. Valicenti-McDermott, McVicar, Cohen, Wershil, & Shinnar / 2008 / Neurology

M. Valicenti-McDermott et al. / 2014 / Journal of Child Neurology

Rome III

Ferguson et al. / 2016 / Brain, Behavior, and Immunity

Peeters, Noens, Philips, Kuppens, & Benninga / 2013 / Journal of Pediatrics

Marler et al. / 2016 / Journal of Autism and Developmental Disorders

Ghalichi, Ghaemmaghami, Malek, & Ostadrahimi / 2016 / World Journal of Pediatrics

Gorrindo et al. / 2012 / Autism Research

Pusponegoro, Ismael, Firmansyah, Sastroasmoro, & Vandenplas / 2015 / Acta Paediatrica (Oslo, Norway : 1992)

Iovene et al. / 2016 / Mycopathologia

M. Valicenti-McDermott et al. / 2015 / Journal of Child Neurology

Kheirouri, Kalejahi, & Noorazar / 2016 / Turkish Journal of Medical Sciences

Ferguson et al. / 2016 / Autism Research : Official Journal of the International Society for Autism Research

Mostafa & Al-Ayadhi / 2015 / Behavioral and Brain Functions : BBF

Gabriele et al. / 2015 / Autism Research : Official Journal of the International Society for Autism Research

Rome III and their own approach

Son et al. / 2015 / PloS One

Safety Monitoring Uniform Report Form

King et al. / 2009 / Archives of General Psychiatry

Safety Monitoring Uniform Report Form and Global Impressions Survey

Anagnostou et al. / 2016 / Jama Psychiatry

Secretin Outcome Survey

Unis et al. / 2002 / Journal of the American Academy of Child & Adolescent Psychiatry

Bramati-Castellarin, Patel, & Drysdale / 2016 / Journal of Bodywork and Movement Therapies

Side Effects Review Form

Nikolov et al. / 2009 / Journal of Autism and Developmental Disorders

The Gastrointestinal Severity Index

James B Adams, Johansen, Powell, Quig, & Rubin / 2011 / BMC Gastroenterology

Santocchi et al. / 2016 / BMC Psychiatry

Ming, Stein, Barnes, Rhodes, & Guo / 2012 / Journal of Proteome Research.

Schneider et al. / 2006 / Journal of Autism and Developmental Disorders

D. W. Kang et al. / 2013 / PLoS ONE

Table S3. Associations Examined Between GI Variable and Other Variables among 144 Studies (%)

Associations examined	
None	17%
Intervention/Treatment	24%
Behavioral/Sensory/Regression/Language/Psychological/IQ	28%
Demographic	13%
Genetic	6%
Outcome group (including age of onset)	46%
Parental/Family History Factors	3%
Microbiota-related	3%
Biological sample	20%
Health-related variable (such as weight)	10%
Diet, food selectivity, weaning	12%
Medications	3%
Vaccine-related variables	1%

Table S4. GI Symptom Estimates, Across Types of Study Samples

	Type of Study Sample	Mean	SD	ANOVA F-value (P-value)
Any GI symptom/ Aggregate of symptoms	Population Based	0.29	0.26	1.80 (0.14)
	Population Based & Clinic Based	0.19	--	
	Clinic Based	0.49	0.23	
	Enriched Risk Sample	0.55	--	
	Other/Unclear	0.52	0.23	
Constipation	Population Based	0.16	0.07	3.03 (0.03)
	Population Based & Clinic Based	0.09	--	
	Clinic Based	0.30	0.15	
	Enriched Risk Sample	0.45	--	
	Other/Unclear	0.22	0.10	
Diarrhea	Population Based	0.21	0.16	0.379 (0.77)
	Population Based & Clinic Based	0.03	--	
	Clinic Based	0.15	0.13	
	Enriched Risk Sample	--	--	
	Other/Unclear	0.18	0.18	
Abdominal Pain/Discomfort	Population Based	0.15	0.12	1.79 (0.51)
	Population Based & Clinic Based	0.05	--	
	Clinic Based	0.13	0.08	
	Enriched Risk Sample	--	--	
	Other/Unclear	0.21	0.16	
Nausea, Vomiting	Population Based	0.06	0.09	0.63 (0.55)
	Population Based & Clinic Based	--	--	
	Clinic Based	0.10	0.06	
	Enriched Risk Sample	--	--	
	Other/Unclear	0.05	0.03	

	Type of Study Sample	Mean	SD	ANOVA F-value (P-value)
Bloating, Flatulence, Gas	Population Based	0.12	0.02	0.55 (0.59)
	Population Based & Clinic Based	--	--	
	Clinic Based	0.12	0.09	
	Enriched Risk Sample	--	--	
	Other/Unclear	0.20	0.20	
Reflux, heartburn, acidic stomach, GERD, spitting up	Population Based	0.21	--	3.88 (0.07)
	Population Based & Clinic Based	--	--	
	Clinic Based	0.07	0.05	
	Enriched Risk Sample	--	--	
	Other/Unclear	0.07	0.05	
Soiling, incontinence, bedwetting	Population Based	0.19	--	0.91 (0.52)
	Population Based & Clinic Based	--	--	
	Clinic Based	0.14	0.09	
	Enriched Risk Sample	--	--	
	Other/Unclear	0.02	--	

CHAPTER 4: PSYCHOMETRIC CHARACTERISTICS OF THE AUTISM SPECTRUM DISORDER GASTROINTESTINAL AND RELATED BEHAVIORS INVENTORY

Coauthors: Luke Kalb[1,2,3], Michelle Landrum[2], Rashelle Musci[1,2], Li-Ching Lee[2,4], M. Daniele Fallin[1,2]

[1]Department of Mental Health, Johns Hopkins Bloomberg School of Public Health; [2]Wendy Klag Center for Autism and Developmental Disabilities, Johns Hopkins Bloomberg School of Public Health; [3]Department of Neuropsychology, Kennedy Krieger Institute; [4]Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health

4.1 Background

Individuals with Autism Spectrum Disorder (ASD) tend to have more gastrointestinal (GI) symptoms than their typically developing counterparts[1–21]. As demonstrated in Chapter 3 of this dissertation, the range of symptom estimates across ASD studies is very wide, in part due to the many approaches used to assess GI symptoms. Children with ASD may have difficulties self-reporting medical symptoms, including GI symptoms and pain. Questionnaires designed to measure GI symptoms in typically developing individuals may not be able to capture GI symptoms in all people with ASD. Questionnaires that have been designed for ASD also have limitation, such as not including mealtime or dietary behaviors or not assessing behavioral symptoms that may indicate GI distress. While a number of GI questionnaires have been developed specifically for ASD, none reported psychometric properties until very recently [22].

This recent study adapted the Autism Treatment Network Gastrointestinal Inventory (ATN-GI Inventory) into a 17-item screener called the AS-ATN GI Signs and Symptoms Inventory-17. The four dimensions (factors) identified in this tool (Retentive, Expulsive, Gas, and Motoric) were able to predict functional constipation, functional diarrhea, and gastroesophageal reflux disease (GERD). The tool had a sensitivity of 84%, specificity of 43%, and positive predictive value of 67% for identifying children with one more of these GI disorders. The advantage of this tool, relative to others, is its inclusion of GI-motoric items such as 'In the last 3 months, did your child appear to feel pain when having a BM?' or 'In the last 3 months, did your child push his abdomen with his/her hands or your hands, push his/ her abdomen against or lean forward over furniture?' Items such as these may be particularly helpful in identifying GI distress in a non- or

hypo-verbal child with ASD. However, this tool had a limited number of items having to do with mealtime or dietary preferences/behaviors, which might be reflective of GI symptoms[22].

Therefore, we were interested in developing a modified questionnaire, the ASD Gastrointestinal and Related Behaviors Inventory (ASD-GIRBI), that included GI signs and symptoms, behavioral items, as well specific items on mealtimes, diet, or eating. We achieved this by drawing on two existing tools, the ATN-GI Inventory and the Brief Autism Mealtime Behavior Inventory (BAMBI), as well as deriving new items. We also evaluate the psychometric characteristics of the ASD-GIRBI.

4.2 Methods

4.2.1. Phase 1: Development of questionnaire

4.2.1.1 Review of the literature

We used the NIH Patient-Reported Outcomes Measurement Information System (PROMIS®) Instrument Development and Psychometric Evaluation Scientific Standards to guide the development of the Autism Spectrum Disorder Gastrointestinal and Related Behaviors Inventory (ASD-GIRBI)[23]. We began developing the tool by reviewing the literature on approaches to assessing gastrointestinal symptoms in epidemiologic studies of ASD (dissertation Chapter 3-published in Autism Research[24]). This review guided our development of the item pool and signaled to us what relevant items were missing from previous autism gastrointestinal questionnaires. A key finding from this review was that no existing tool assessed GI symptoms, mealtime behaviors, as well as other behavioral systems (e.g. aggression, self-injurious behavior, sensory sensitivities) that could signal GI distress in a hypo/non-verbal child with ASD.

4.2.1.2. Design of Item Pool

With permission from the authors, we extracted items from two existing autism tools to begin developing the item pool: The ATN-GI[25] and the BAMBI[26–28]. The ATN-GI Inventory was developed by pediatric gastroenterologists from the Autism Speaks-Autism Treatment Network, and was designed to assess for functional constipation, functional diarrhea, and GERD[22]. The BAMBI, an 18-item caregiver-report questionnaire, was designed to evaluate mealtime behaviors in children with ASD and has been shown to have good internal consistency, high test-retest reliability, and strong criterion-related validity[26]. We also added *de novo* items to our item pool, based on our review of the literature, qualitative interviews of children with ASD and their parents, and feedback from an expert panel, described below.

4.2.1.3 Interviews with parents/individuals with ASD

Prior to, during, and following the development of our initial GI inventory, we held one-on-one qualitative interviews with parents of children with ASD. In some cases, their children would join the interview. Individuals were eligible to participate if they were the parent or caregiver of a child with ASD that had a history of GI symptoms during the ages of 3-18. Individuals with ASD who had GI symptoms during these ages were also eligible. We intentionally did not define 'gastrointestinal symptoms' or provide examples, unless a potential participant asked for clarification, because we wanted to capture all possible GI-related issues to ensure our item pool covered all relevant domains.

We used social media outlets (e.g. Facebook), email listservs, and website postings to recruit individuals from advocacy groups and other ASD-centered groups. We asked individuals to share the post with anyone who might be interested, with the aim of attracting a diverse group of

individuals. Participants received a $10 Amazon gift card for participating, as well as access to a private (for study participants) free webinar, during which we will explain the study findings and how the results are being used.

Interviews took 30-45 minutes and were held either in person in a private location such as the participant's home, or through a video/audio conversation using the Zoom Video Conferencing Platform. Interviews were audio recorded and transcribed, and notes were also taken during each interview. One-on-one qualitative interviews were carried out instead of focus groups because of difficulties in scheduling multiple parents together.

Participants were asked questions such as 'What are the gastrointestinal issues your child currently struggles with or has struggled with in the past? What are things you notice about your child when they are having GI symptoms/distress? What are some signs/behaviors that you see? What areas related to GI issues have affected your family or your child's functioning?' We probed participants to expand on their experiences, provide examples, or clarify any remarks. The exact language that was used was adapted to each participant. Simpler language was used when also interviewing the child, especially in the case of a co-morbid intellectual disability or communication impairment.

4.2.1.4 Feedback/revisions from experts

After the initial development of the item pool, a panel of experts was consulted on whether items or domains were missing from the tool and whether language or directions were unclear. The panel consisted of experts in ASD, gastroenterology, psychometrics, epidemiology, and public

health. Items were added, removed, or edited for clarity based on feedback from this expert group.

4.2.1.5 Cognitive Debriefing

Lastly, cognitive interviews were carried out. During this in-person session, parents completed a sample of items on the tool and were asked to provide feedback on the clarity of wording, the relevance of the item, what construct the item conjured for them, and whether the items were upsetting or insensitive. Revisions were made to the questionnaire based on these interviews.

4.2.2 Phase 2: Administering the Questionnaire

4.2.2.1 Sample

The study population for phase 2 consisted of a registry of parents with a child with ASD at Kennedy Krieger Institute (KKI), a center that combines research, clinical service, therapeutic day programs, and training programs for children with developmental disabilities and disorders of the brain, spinal cord, and musculoskeletal system. These parents had previously consented to being contacted for research purposes. We sent an invitation to the 2,335 families whose child had a confirmed diagnosis of ASD. If parents were interested in joining the study, they were provided a Qualtrics survey link, which included a consent form, as well as the ASD-GIRBI and the Child Behavior Checklist (described below). All data collection happened electronically. Five hundred thirty seven (537) families consented to join the study, of which 444 (83%) completed both the ASD-GIRBI and the Child Behavior Checklist. Parents with a child with ASD between the ages of 3-18 were eligible to join the study, regardless of the child's experience with GI symptoms.

4.2.3. Ethical Considerations

For the qualitative, phase 1, portion of the study, parents participating by themselves (without their child present) consented to the study. Individuals with ASD younger than 18 who participated with their parents needed parental permission and provided assent. Individuals with ASD 18 or older consented for themselves, unless a caregiver deemed they were not able to, in which they case the parent provided permission, and we relied on assent from the individual with ASD.

For the online registry phase, the parent or primary caregiver was the informant for all children and young adults with ASD (ages 3-17). Parents completed a consent form online prior to completing the study questionnaires. Participants were provided a phone number and email address to contact study staff, as well as contact information for the IRB.

Both phases of this study were approved by the local institutional review board.

4.2.4. Measures

4.2.4.1 CBCL

Participants from the KKI research registry completed either the Child Behavior Checklist (CBCL) 1.5-5 or the CBCL 6-18, depending on their age. The CBCL is a reliable, valid 99-item questionnaire completed by the parent/caregiver who spends the most time with the child. The CBCL can be completed at home in 10-20 minutes. For each problem item, such as "disturbed by any change in routine", parents are asked to rate how true each item is for their child is: not true (within past 6 months), somewhat or sometimes true (within past 6 months), or very true or

often true (based on the past 2 months)[29–31]. Each syndrome domain can be scored as being in the normal range, in an area of concern but not considered very deviant, or in the clinical range, based on scores from a national normative sample. We derived a score from each of the following CBCL domains: Anxiety/Depression, Emotionally Reactive, Somatic Complaints, Withdrawn, Attention Problems, Aggressive Behavior, and Sleep Problems.

4.2.4.2. GI questionnaire

The initial GI questionnaire, prior to revision following the psychometric analysis, consisted of 56 core items, with an additional 8 follow-up questions if certain core items were endorsed. Only the 56 core items were used in the psychometric analysis. The first section asked parents to report physical/mental health diagnoses (n=14 items). The core set of questions included four sections: 1) 11 gastrointestinal symptoms, with follow-up questions on symptom duration (within the last 3 months only, 3-5 months, 6-11 months, 1 year or longer, not sure) and GI symptom association with bowel movements, eating, and weight (2) frequency of bowel movements and stool consistency (Bristol stool chart[32]) in addition to seven items on bathroom/toileting behaviors, 3) 20 items on mealtime and dietary behaviors , and 4) 13 other behaviors (e.g. unexplained irritability, agitation, aggression, or screaming; chewing on shirts, eating non-edible objects; pointing to stomach/tummy as if in pain). Parents were also asked about medications their child was taking, how GI symptoms impacted their child's functioning, and their confidence in accurately assessing their child's pain level. The full questionnaire can be found in the Appendix.

4.2.5. Analysis

4.2.5.1. Phase 1 Qualitative Interviews

Directed content analysis was carried out using a general inductive approach for each qualitative interview[33,34]. Major themes were identified and summarized. In addition, particular examples of GI symptoms, mealtime/dietary behaviors, and other signs/behaviors that indicate GI distress in children with ASD were extracted for inclusion in the questionnaire.

4.2.5.2 Phase 2 Statistical Analysis

We carried out a psychometric assessment of the ASD-GIRBI by performing exploratory factor analysis, assessing the reliability of the tool with Cronbach's alpha, and assessing convergent validity. The 56 core GI questionnaire items were used for psychometric assessment (GI symptoms, toileting behavior and bowel movements, mealtime and dietary behaviors, other behaviors). Questions only answered by a subset of participants, for example, questions about the duration of GI symptoms, were not included in the psychometric analysis. All data cleaning and analyses were performed in R Studio version 1.1.383 (R version 3.4.3). Because of insufficient sample size for children ages 3-5, we only carried out the factor analysis and assessments of reliability and convergent validity in the group 6-17 years of age.

4.2.5.3 Factor Analysis

We performed exploratory factor analysis (EFA) on the individuals ages 6-17 in order to determine the factor structure of the GI questionnaire. We first dropped items endorsed by <10% of individuals, items that decreased the scale's internal consistency (Cronbach's alpha), and items that did not load onto a factor (score <0.30). No two items had a pairwise correlation greater than 0.70, suggesting we did not have redundant items. We followed the 5-step procedure recommended by Costello and Osborne[35]. The *Psych* package in R was used to perform parallel analysis of principal components using minimum residuals, in order to extract

factors. We chose *oblimin rotation* (oblique), allowing factors to be correlated to each other. After dropping items from the measure based on the EFA findings, we evaluated the factor structure using fit indices, including the root mean square error of approximation (RMSEA), the comparative fit index (CFI), and the Tucker-Lewis index (TLI). A CFI and TLI of ≥0.9 is considered an acceptable fit and a RMSEA <0.10 is considered a good fit[36,37].

We calculated factor scores by taking the sum of the number of items endorsed by the person for each factor. For example, if a factor consisted of 7 items, individuals who endorsed all 7 items would receive a factor score of 7, while those who did not endorse any of the items would receive a score of 0.

4.2.5.3 Reliability

We assessed the reliability of the total scale and of each item via Cronbach's alpha and item-rest correlations, respectively. Dropped items that decreased the scale's internal consistency (Cronbach's alpha) were omitted.

4.2.5.3 Convergent validity

Convergent validity is 'evidence of similarity between measures of theoretically related constructs'. This was assessed by estimating associations between factor scores and subscales on the CBCL, parent-report GI diagnoses, and parent-report functioning impairment due to GI symptoms[38]. Associations with directions and magnitudes as expected suggest good convergent validity.

4.3 Results

4.3.1. Participant Characteristics

4.3.1.1 Phase 1

We carried out 12 qualitative interviews with parents of children with ASD, two of which also included the child. Ten of the interviews were with the mother, and the remaining two were with the father. All but three interviews were in reference to children still under the age of 18. Eleven of the individuals with ASD were male, and one was female.

4.3.1.2 Phase 2

Of the 2,335 study invitations sent to KKI registry families, 537 participants consented to complete the survey. Individuals who did not complete both the GI tool as well as the CBCL were excluded (n=93), leaving 444 children (Figure 1). The majority (75%) of these children were between the ages of 6-17 years old (Table 1). Over 90% of participants who completed the survey on behalf of their child were mothers and highly educated, with 89-90% having some college/AA education or greater. Children identified as mostly male (83% in 3-5 year olds, 78% in 6-17 year olds). Just over half (52-59% of participants were white, 18-25% were black or African-American, 14-16% multiracial, 7-8% Asian, and 7-9% Hispanic/Latino). The most common medical diagnoses were allergies/asthma, gastrointestinal disorders, and sleep disorders. The most common psychiatric/developmental disorders endorsed were sensory processing disorder, anxiety, panic, or phobia disorder, ADD/ADHD, intellectual disability, and obsessive-compulsive disorder (Table 2).

Figure 1. Flow Chart of Study Participant Recruitment

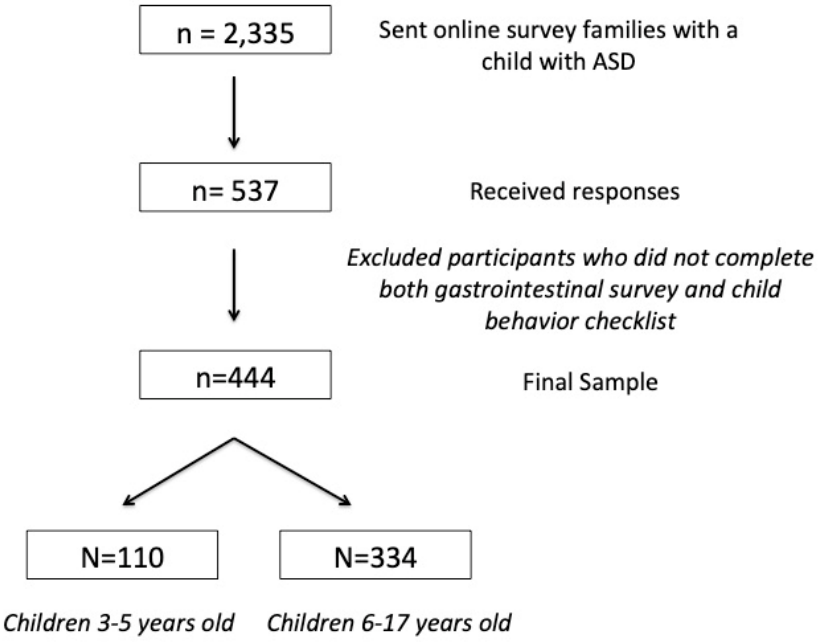

Table 1. Demographic Characteristics of Study Participants (% or mean (SD))

	Ages 3-5 years (n=110)	Ages 6-17 years (n=334)
Child age (years)	4.3 (0.75)	9.5 (3.09)
Respondent relationship to child		
Mother	93%	92%
Parent education level		
High school graduate/GED or below	12%	11%
Some college/AA education	32%	19%
College/AA degree	25%	32%
Graduate education	32%	39%
Child biological sex		
Female	22%	25%
Male	78%	75%
Child gender identity		
Female	17%	21%
Male	83%	78%
Non-binary, gender-queer, gender-fluid, or transgender	0%	1%
Child race/ethnicity		
White	52%	59%
Black/African-American	25%	18%
Multiracial	14%	16%
Asian	8%	7%
Hispanic/Latino	7%	9%

Table 2. Self-reported Psychiatric and Medical Diagnoses of Study Participants

	Ages 3-5 years (n=110)	Ages 6-17 years (n=334)
Psychiatric and Medical Diagnoses		
Any gastrointestinal disorder	19%	20%
Seizure/Epilepsy disorder	2%	7%
Intellectual Disability	26%	31%
ADD/ADHD	12%	55%
Sensory processing disorder	50%	49%
Anxiety, panic, or phobia disorder	15%	39%
OCD	7%	13%
Tic/Tourette's disorder	2%	3%
Depression	1%	8%
Bipolar disorder	1%	1%
Sleep disorder	8%	16%
Autoimmune disorder	2%	3%
Allergies or Asthma	27%	39%
Other	13%	18%
Gastrointestinal Disorder		
Acid Reflux / GERD / Rumination	8%	6%
Constipation	6%	6%
Food Intolerance / Sensitivity / Celiac Disease	2%	1%
Encopresis	0%	2%
Medications		
Antidepressant	2%	12%
Antipsychotic / Tranquilizer	3%	6%
Anti-anxiety medication (e.g. benzodiazepine or hypnotics)	1%	14%
Mood stabilizer	0%	10%
Stimulant	2%	19%
Anticonvulsant	1%	4%
Prescription sleep medication	5%	10%
Hypotensive medication	1%	5%
Other	11%	22%

4.3.2. Exploratory Factor Analysis

Exploratory factor analysis was carried out only among the 6-17 year olds (n=334), because of an insufficient sample size in the younger group (n=110). Two items were dropped for being endorsed by <10% participants (BM frequency >3 per day and pushing on their own chest/neck/throat). Seven items were removed for lowering the scale's overall Cronbach's alpha (reflux or heartburn, having constipation, i.e. type 1 & 2 on Bristol Stool Chart, having diarrhea, i.e. types 5, 6, and 7 on Bristol Stool Chart, inflexibility about mealtimes routines (e.g. times for meals, place settings, seating arrangements, meal locations), preferring only sweet foods, being on a special diet (e.g. gluten free, casein free, FODMAPS, GAPS), and drinking lots of water with meals. Finally, twelve items were dropped for not loading onto a factor (difficulty falling/staying asleep, having ideal stool consistency, i.e. types 3 & 4 on Bristol Stool Scale, refusing foods that require lots of chewing, chewing on non-edible objects, abdominal swelling or distention, avoiding wearing tight clothing or clothing with waistbands, frequent clearing of throat, swallowing, coughing, gagging, choking, or throat sounding wet or gurgly, unexplained irritability, agitation, aggression, or screaming, bowel movement frequency <3 per week, rushing to the bathroom for a bowel movement, moaning or groaning for no apparent reason, alternating diarrhea and constipation). In total, the number of core items dropped from 56 to 35 items.

A seven-factor solution emerged as the best fit for the data (Factor 1: constipation & pain during bowel movements, Factor 2: doesn't want food at times, Factor 3: Particular with foods, Factor 4: abdominal pain/vomiting/gassiness/diarrhea, Factor 5: incontinence/soiling/wetting the bed, Factor 6: aggressive/disruptive at mealtimes, Factor 7: Other behaviors). Factor loadings for each questionnaire item can be found in Table S2. Table S3 summarizes correlations between

factor scores. Pairwise correlations between factor scores were weak, ranging from 0.04 (factors 4 and 6) to 0.34 (factors 1 and 4). The items belonging to each factor are shown in Box 1. The distribution of factor scores can be found in Figure S1. The RMSEA of the scale was 0.06, indicating a good fit, and the TLI was 0.83. In total, the seven factors accounted 39% of the scale, with each factor accounting for 3-7% of the variance.

Box 1. Factor Names and Items Loading onto each Factor of the ASD-GIRBI[c]

Factor 1: Constipation & Bowel Movement Pain	Factor 2: Doesn't want food at times	Factor 3: Particular with foods	Factor 4: Abdominal Pain / Vomiting / Gassiness / Diarrhea	Factor 5: Incontinence / Soil / Wet bed	Factor 6: Aggressive/ Disruptive at Mealtimes	Factor 7: Other behaviors
Bloating	Turns their face or body away from food	Not willing to try new foods	Abdominal pain	Incontinence / Lack of voluntary control or urination or defecation	Cries or screams during mealtimes	Applying pressure to their abdomen by pushing on it or leaning on furniture
Constipation	Closes their mouth tightly when food is presented	Does not accept/ prefer a variety of foods	Nausea, vomiting, or retching/dry heaving	Fecal Retention / complete elimination of stool	Is aggressive during mealtimes (hitting, kicking, scratching others)	Unusual movements such as thrusting jaw, tilting head, arching back, or twisting neck/body
Fecal Retention / complete elimination of stool	Spits out food that they have put in their mouth	Prefers same foods at each meal	Bloating	Stiffen their legs or squeeze their bottom and legs together when they felt need to have a BM	Displays self-injurious behavior during mealtimes (hitting self, biting self)	Gritting teeth, wincing, or grimacing for no apparent reason
Appear to feel pain when having a BM	Stops eating after just a little food	Prefers food prepared particular way	Flatulence/gas	Stain or soil underwear	Is disruptive during mealtimes (pushing/throwing utensils or food)	
Stiffen their legs or squeeze their bottom and legs together when they felt need to have a BM	Does no remain seated at the table until the meal at finished	Avoides eating a particular type of food group	Diarrhea	Wet the bed	Biting themselves, putting their fist in their mouth, or hurting themselves in other ways	
Become more active after passing a stool	Cries or screams during mealtimes	Strongly prefers certain types of food colors, textures, temperatures	Pointing to stomach/tummy as if in pain			
Become less irritable after passing a stool			Direct vocalizations of pain (e.g. "tummy hurts" "stomach pain")			

[c]Items with a factor loading greater or equal to 0.30 were assigned to a factor. Items could load onto more than one factor.

4.3.3. Reliability

Table 3 summarizes the alpha and item-rest coefficients and item prevalences for the final 35 items in the GI tool. The Cronbach's alpha for individual items was 0.84-0.54. The overall alpha for the scale was 0.85.

4.3.4. Convergent validity

Children with a parent-reported diagnosis of any GI disorder were significantly more likely to have higher clinical scores on factor 1 (constipation & bowel movement pain), factor 3 (particular with foods), factor 4 (abdominal pain/vomiting/gassiness/diarrhea), factor 5 (incontinence/soiling/wetting the bed), and factor 7 (other behaviors) ($p<0.05$). Children with a parent-reported diagnosis of acid reflux, GERD, or rumination were significantly more likely to have higher clinical scores on factor 3 (particular with foods) and factor 4 (abdominal pain/vomiting/gassiness/diarrhea) ($p<0.05$). Children with a parent-reported diagnosis of constipation were significantly more likely to have higher clinical scores on factor 1 (constipation and bowel movement pain) and factor 3 (particular with foods) (Table 4).

Correlations were calculated between factor scores and CBCL subscales (Table S4). Correlations between factor scores and the CBCL subscales were weak on average. The highest correlations were between 0.30-0.35, with the following subscales: aggressive behavior, somatic complaints, social problems, and thought problems. Factors 3 (particular with foods) and 5 (incontinence/soiling/wetting the bed) were not significantly associated with any CBCL subscales.

We estimated the mean difference in clinical factor scores across levels of functional impairment due to GI symptoms (missed school/was late, missed social/family activities, trouble falling/staying asleep). Almost all factor scores were significantly associated with these three types of functional impairment ($p<0.05$) (Table S5).

Lastly, for the factors that were significantly associated with a parent-report GI diagnosis, we calculated the sensitivity, specificity, and area under the curve (AUC) of Received Operating Characteristic (ROC) Curves, in order to assess how factor scores predict self-reported GI diagnoses. We were primarily interested in maximizing sensitivity (correctly identifying children who have GI symptoms), so we set a threshold score of 1, meaning individuals scoring at least a 1 on a factor were classified as screening 'positive'. Factors 1, 3, 4, and 5 all had relatively high sensitivities for detecting any GI disorder at a cut off of 1 point (89%, 91%, 86%, and 80%, respectively) (Table 5). Factor 7 had a low sensitivity for any GI disorder (56%). We chose Factor 1 as the optimal screener for any GI disorder, because it had a high sensitivity (89%), and a higher specificity than factor 3 (31% vs. 6%), and a higher AUC than factor 3 (69% vs. 51%). Factors 1 and 3 both had high sensitivity in detecting constipation (both 95%, though Factor 1 had a higher specificity than factor 3 (24% vs. 7%) as well as a higher AUC (71% vs. 55%). Therefore, Factor 1 was also the optimal predictor of constipation. Factors 3 and 4 both had relatively high sensitivities for predicting acid reflux/GERD/rumination (85% and 90%, respectively). However, Factor 4 had a higher specificity (26% vs. 6%) as well as a higher AUC (65% vs. 58%), so it was the optimal screener.

Table 3. Cronbach's Alpha for Total ASD-GIRBI Scale and Individual Items, among Children 6-17 Years

	Cronbach's alpha for Total Scale= 0.85		
Item	**Item-Rest Correlation**	**Overall alpha if item dropped**	**Item Prevalence**
Abdominal pain	0.51	0.85	45%
Nausea, vomiting, or retching/dry heaving	0.37	0.85	23%
Bloating	0.48	0.84	27%
Flatulence or gas	0.50	0.85	61%
Diarrhea	0.41	0.85	39%
Constipation	0.45	0.85	58%
Incontinence / Lack of voluntary control or urination or defecation	0.34	0.85	19%
Fecal Retention / complete elimination of stool	0.36	0.85	19%
Appear to feel pain when having a BM	0.46	0.85	42%
Stiffen their legs or squeeze their bottom and legs together when they felt need to have a BM	0.56	0.85	39%
Stain or soil underwear	0.46	0.85	48%
Wet the bed	0.33	0.85	29%
Become more active after passing a stool	0.53	0.85	49%
Become less irritable after passing a stool	0.56	0.85	56%
Turns their face or body away from food	0.49	0.85	44%
Closes their mouth tightly when food is presented	0.47	0.85	28%
Spits out food that they have put in their mouth	0.51	0.85	35%
Stops eating after just a little food	0.42	0.85	50%
Remains seated at the table until the meal at finished	0.32	0.85	36%
Cries or screams during mealtimes	0.34	0.85	18%
Is aggressive during mealtimes (hitting, kicking, scratching others)	0.40	0.85	14%
Displays self-injurious behavior during mealtimes (hitting self, biting self)	0.37	0.85	10%

Item	Item-Rest Correlation	Overall alpha if item dropped	Item Prevalence
Is disruptive during mealtimes (pushing/throwing utensils or food)	0.43	0.85	18%
Is willing to try new foods	0.42	0.85	44%
Accepts or prefers a variety of foods	0.41	0.85	38%
Prefers the same foods at each meal	0.34	0.85	84%
Prefers food prepared in a particular way (e.g. eats mostly fried foods, cold cereals, raw vegetables)	0.40	0.85	75%
Prefers to avoid eating a particular types of food group (e.g. vegetables, meats, dairy)	0.40	0.85	73%
Strongly prefers certain types of food colors, textures, or temperatures	0.46	0.85	74%
Applying pressure to their abdomen by pushing on it or leaning on furniture	0.49	0.85	30%
Unusual movements such as thrusting jaw, tilting head, arching back, or twisting neck/body	0.40	0.85	19%
Gritting teeth, wincing, or grimacing for no apparent reason	0.44	0.85	23%
Biting themselves, putting their fist in their mouth, or hurting themselves in other ways	0.34	0.85	16%
Pointing to stomach/tummy as if in pain	0.39	0.85	16%
Direct vocalizations of pain (e.g. “tummy hurts” “stomach pain”)	0.40	0.85	38%

Table 4. Mean Differences in ASD-GIRBI Factor Scores by Parent-Report GI Diagnoses

	Any gastrointestinal disorder	Acid Reflux / GERD / Rumination	Constipation
Factor 1 - Constipation & Bowel Movement Pain	3.32 vs.1.89*	3.05 vs.2.4	3.8 vs.2.35*
Factor 2 - Doesn't want food at times	1.97 vs.1.98	2.2 vs.2.04	2 vs.2.05
Factor 3 - Particular with foods	3.77 vs.3.75*	4.15 vs.3.76*	4.1 vs.3.77*
Factor 4 - Abdominal Pain / Vomiting / Gassiness / Diarrhea	3.02 vs.1.92*	3.3 vs.2.27*	2.4 vs.2.33
Factor 5 - Incontinence / Soil / Wet bed	2.11 vs.1.1*	1.4 vs.1.41	1.85 vs.1.39
Factor 6 - Aggressive/ Disruptive at Mealtimes	0.74 vs.0.65	0.85 vs.0.75	0.9 vs.0.74
Factor 7 - Other behaviors	0.92 vs.0.54*	1.05 vs.0.66	0.85 vs.0.67

*Signify mean differences with a $p<0.05$, according to a t-test.

Table 5. Sensitivity, Specificity, and Area Under the Curve of ASD-GIRBI Clinical Factor Scores in Predicting Parent-Report Gastrointestinal Disorder Diagnoses

		Sensitivity[a]	Specificity[a]	Area Under the Curve
Any gastrointestinal disorder[b]	Factor 1 Score	89%	31%	69%
Any gastrointestinal disorder	Factor 3 Score	91%	6%	51%
Any gastrointestinal disorder	Factor 4 Score	86%	31%	66%
Any gastrointestinal disorder	Factor 5 Score	80%	42%	69%
Any gastrointestinal disorder	Factor 7 Score	56%	62%	61%
Constipation[b]	Factor 1 Score	95%	24%	71%
Constipation	Factor 3 Score	95%	7%	55%
Acid Reflux / GERD / Rumination	Factor 3 Score	85%	6%	58%
Acid Reflux / GERD / Rumination[b]	Factor 4 Score	90%	26%	65%

[a]Sensitivity and specificity were calculated for a factor score cut-off of 1.
[b] These factors were chosen as optimal screeners for GI diagnoses.

4.4 Discussion

In this study we developed a parent-report screener for GI symptoms in children with ASD. This tool included GI signs and symptoms, as well as items on mealtime and dietary behaviors, and other less-specific behaviors, which could indicate GI distress in a non/hypo-verbal child with ASD. This 35-item screener was derived from two existing tools: The ATN GI Inventory and the BAMBI, and also included new items. Two factors on the screener detected parent-report of any GI disorder, constipation, and acid reflux/GERD/rumination with very high sensitivity (89%, 90%, and 95%).

We found the ASD-GIRBI to be an internally consistent for detecting GI symptoms in children with ASD ages 6-17 years old. The Cronbach's alpha was good for all items (0.84-0.84) and for the whole scale (0.85). Exploratory factor analysis identified a seven-factor model. Factor 1 included items having to do with constipation. Bloating, pain during bowel movements, fecal retention/incomplete elimination, becoming more active or less irritable after a stool, or squeezing legs/bottom together when needing to go to the bathroom, are all items that don't directly measure constipation, but all indicate that a child is in distress/pain, is withholding stool, and doesn't want to use the toilet. The 95% sensitivity of this factor score in identifying diagnosis of constipation is therefore not surprising. Factor 2 had to do with a child not wanting food at times. Indicators of this included the child turning away from food, closing their mouth tightly when food is presented, spitting out food, stopping eating after just a little food, crying or screaming during a mealtime, or not remaining seated for the duration of a meal. This factor was not significantly associated with any GI disorder, though it was weakly associated with aggressive behavior on the CBCL, and associated with missed school or being late, missing

social or family activities, and trouble falling or staying asleep. Factor 3 included items reflecting food preferences and aversion. This factor had quite high sensitivity in identifying individuals with any GI disorder, or with constipation, or acid reflux/GERD/rumination. This likely reflects the strong comorbidity between food preferences/aversions and having GI symptoms. Factor 4 was more difficult to interpret, as it included abdominal pain, nausea or vomiting, bloating, flatulence, or gas, and diarrhea, and also two indicators of pain (pointing to stomach/tummy or direct verbalizations). This factor was chosen to screen for individuals with acid reflux, as it had a sensitivity of 90% and a specificity of 26%. Factor 5 included items having to do with incontinence, soiling, or wetting the bed. It included items in common with the constipation factor (factor 1), which is expected. Factor 6 had to do with aggressive or disruptive behavior during mealtime, including crying, screaming, being aggressive, self-injurious, and disruptive, for example, pushing or throwing utensils or food. This factor was not significantly associated with any GI disorder, though like factor 2 (not wanting food at times), it was still associated with aggressive behavior and thought problems on the CBCL, and with trouble falling or staying asleep. Lastly, factor 7 included 3 non-specific behaviors (e.g. 'applying pressure to their abdomen by pushing on it or leaning on furniture'), which could indicate GI distress in a non- or hypo-verbal child with ASD. While we found strong and significant associations between factor scores on the ASD-GIRBI and parent-reported diagnoses of GI disorders, the association between the items in factor 7 and having any GI diagnosis was notable. However, it was not useful as a screener in ROC analyses, as will be described in greater depth below.

Correlations between factor scores and the CBCL subscales were weak to moderate on average. However, all correlations were positive in magnitude, meaning having worse GI or related symptoms were associated with problems on the CBCL. The highest correlations we found were between factor 1 (constipation and bowel movement pain) and somatic complaints (r=0.34), factor 2 (not wanting foods at times) with aggressive behavior (r=0.34), factor 4 (abdominal pain/vomiting/gassiness/diarrhea) and somatic complaints (r=0.54) and social problems (r=0.34). Factor 6 (aggressive/disruptive at mealtimes) was associated with aggressive behavior (r=0.42) and thought problems (r=0.34). Lastly, factor 7 (other behaviors) was correlated with thought problems (r=0.34). Given that none of the CBCL subscales are measuring GI symptoms, we would not expect to find very strong associations. Seeing moderate, positive correlations with somatic complaints and aggressive behaviors, for example, provide some evidence of convergent validity.

Lastly, every single factor on the ASD-GIRB was significantly associated with greater levels of functional impairment at school, in social settings, or with sleeping, or in many cases, all of the above. This not only provides further evidence of convergent validity of our tool, but also highlights how GI and related symptoms may impact a child's functioning across multiple settings and may influence other health comorbidities.

In ROC analyses, using a cut-off score of 1, we found that factor 1, having to do with constipation and bowel movement pain) detected any parent-reported GI diagnosis and constipation with sensitivities of 89% and 95%, respectively. Factor 4 (abdominal pain/vomiting/gassiness/diarrhea) detected parent-report diagnosis of acid

reflux/GERD/rumination with a sensitivity of 90%. We were particularly interested in developing a sensitive screener, since children with GI symptoms who are able to self-report complaints to their parents are likely to be identified without the need for a GI questionnaire. Therefore, we found the low specificities (ranging from 26% to 31%) acceptable. Factor 3, being particular with foods, was also highly sensitive for any GI disorder or constipation (>90% sensitivity), but the specificity of this factor was <10%. This is not surprising, as food preferences may reflect other issues such as sensory sensitivities, independent of GI symptoms. The high sensitivity of this factor reflects the strong association between having GI symptoms and dietary preferences/aversions. It is striking that in our analysis of 6-17 year old children, 84% prefer the same foods at each meal, 75% prefer foods prepared a particular way, 73% avoids eating a particular type of food group, and 74% strongly prefer certain types of food colors, textures, or temperatures. This is in contrast to 44% who are willing to try new foods and 38% who accept or prefer a variety of foods.

We expected non-specific behaviors to be more predictive of GI diagnoses. Following psychometric analysis of our tool, we removed seven non-specific items because of either low endorsement (n=1) or not loading onto a factor (n=6). Three items remained in Factor 7: 1) applying pressure to their abdominal by pushing on it or leaning on furniture, 2) unusual movements such as thrusting jaw, tilting head, arching back, or twisting neck/body, and 3) gritting teeth, wincing, or grimacing for no apparent reason. Three other behavioral items loaded onto two other factors. Having at least one of these Factor 7 items captured 56% of the constipation diagnoses, with a specificity of 62%. Behaviors including pointing to the stomach/tummy as if in pain (factor 4), direction vocalizations of pain (factor 4), becoming more

active/less irritable after passing a stool (factor 1), and stiffening/squeezing legs or bottom together (factor 1) were included in the final screener, and were predictive of GI disorders, suggesting that these behavioral items do play a role in helping with the identification of GI disorders in children with ASD. Only 40% of parents reported their child either points to their stomach/tummy if in pain or directly verbalizes they are in pain, meaning the majority must rely on something else to detect this, or are unsure when their child is in pain. Indeed, among the parents of children 6-17 years old, 12% reported they were not confident at all in assessing their child's GI pain, 23% reported they were slightly confident, 56% were fairly confident, and only 18% were completely confident.

There were a number of limitations to this study. Perhaps most importantly, we did not have a gold-standard measure of GI symptoms to compare our tool against. We relied on parent-report of GI diagnoses to calculate sensitivity and specificity. Ideally, every child in our study would have been assessed for a GI disorder by a physician; however, this was not feasible. Even if feasible, the more critical issue is that there is currently no gold-standard approach to assessing for GI disorders in this population. Even a trained gastroenterologist may misclassify someone as not having a GI disorder, if the patient or a proxy respondent is not able to accurately report symptoms. This is especially the case for 'functional GI disorders', which are GI conditions that cannot currently be attribute to another medical condition[39]. In fact, rates of parent-report of GI diagnoses in this study may be an underestimates if children with GI disorders haven't been recognized as having one. For these reasons, we focused on measures of consistency. Another limitation of this study is that due to an insufficient sample size in children less than six years old, we were only able to carry out the psychometric analysis among children 6-17 years old.

The performance of individual items and the factor model would likely differ in this younger group. Next, our response rate was relatively low, at 23%. This is not uncommon in research, particularly internet-based research, but likely means our sample is not representative of all children with ASD. Similarly, the frequency of GI and related behaviors from this study should not be taken as representative of the underlying ASD population, as parents with children who have more severe GI symptoms are more likely to participate in studies like this, though we didn't have data to confirm this. We unfortunately did not have demographic or clinical information on families who did not wish to enroll in the study, so we cannot assess how similar or different the non-participants are from our study sample.

Our study also had a number of strengths. First, we developed a new parent-report tool for detecting GI symptoms among children with ASD, using two existing tools as well as new items, derived from qualitative interviews with parents of children with ASD and review by an expert panel. Therefore, we feel confident that this tool has a high degree of content validity. We also followed PROMIS guidelines for instrument development and psychometric evaluation. This study is only the second to carry out a psychometric assessment of a GI assessment tool for individuals with ASD. The first psychometric study came out in 2018, was also based off of the ATN-GI Inventory, and has items in common with the tool analyzed in the present study[22].

Factors 1 and 4, which together consist of 13 items, are the only factors necessary to identify children with any GI disorder, constipation, or acid reflux with high sensitivity. However, the other items on this questionnaire may provide useful and contextual information for parents, researchers, and clinicians alike. In addition to including questions on GI symptoms, mealtime

and dietary behaviors, and other non-specific GI items, we also included three questions on how a child's GI symptoms affect their functioning. While not part of the psychometric analysis presented here, items on duration of symptoms, how symptoms change following a bowel movement, eating, as well as which medications children are taking also provide useful information. The parent-report nature of this tool is useful for epidemiologic studies because it is inexpensive and easy to administer to study participants.

The next steps for this project include carrying out item response theory to better understand which specific items within each factor are especially informative and useful in predicting GI disorders. Latent class analyses of individuals in this study could also be performed to identify subgroups of children with ASD with particular types of GI and related symptoms. Although not without limitations, physician assessment would be very useful to achieve a more accurate picture of which children have an underlying GI disorder. Lastly, other independent ASD samples are needed to assess the performance of this questionnaire.

While the need for more accurate assessment of GI issues in ASD has started to receive increasing attention, there is still much work to be done. Parent- and self-report tools need further validation efforts in diverse groups of people with ASD. Tools developed for research purposes, such as this one, may provide useful in clinical settings as well. Individuals with ASD deserve to have their GI symptoms recognized and treated with the same quality as typically developing individuals do.

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Figure S1. Distribution of ASD-GIRB Factor Scores among Children 6-17 Years Old

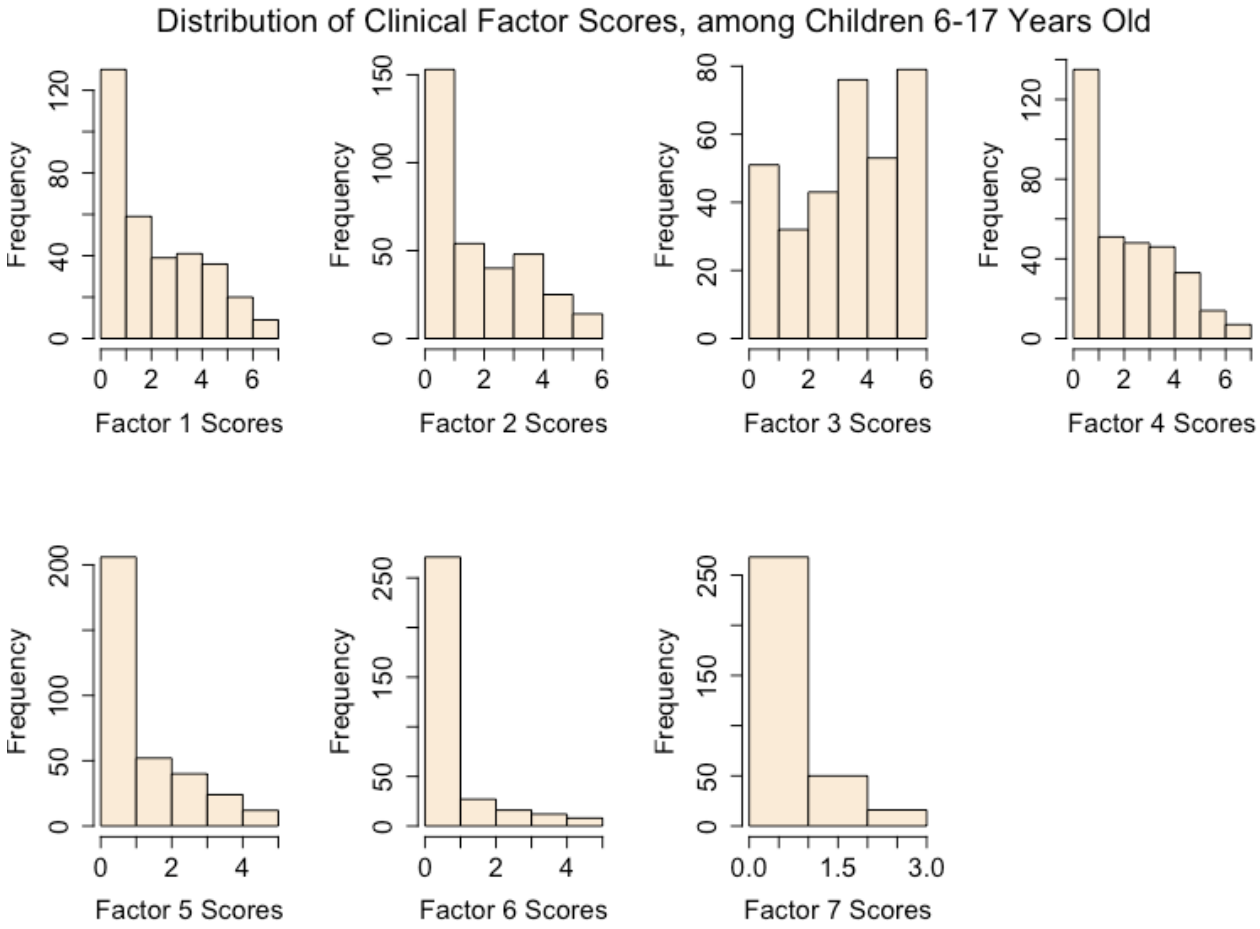


Table S1. Gastrointestinal Symptoms, Bathroom-Related Behaviors, Mealtime/Dietary Behaviors, and Other Behaviors in Last three Months, Stratified by Age (%)

	Ages 3-5 years (n=110)	Ages 6-17 years (n=334)
Gastrointestinal Symptoms		
Abdominal pain	25%	40%
Nausea, vomiting, or retching/dry heaving	24%	22%
Reflux or heartburn	15%	14%
Abdominal swelling or distention	12%	13%
Bloating	22%	24%
Flatulence or gas	53%	59%
Diarrhea	46%	37%
Constipation	64%	54%
Alternating diarrhea and constipation	33%	22%
Incontinence / Lack of voluntary control or urination or defecation	24%	18%
Fecal Retention / complete elimination of stool	22%	16%
Any of the above	83%	83%
Bristol Stool Chart – Constipation (Types 1 & 2)	53%	51%
Bristol Stool Chart – Diarrhea (Types 5,6,7)	34%	25%
Bristol Stool Chart – Ideal (Types 3 &4)	49%	57%
Average BM more than 3 per day	5%	3%
Average BM less than three per week	15%	17%
Bathroom-Related Behaviors		
Appear to feel pain when having a BM	40%	36%
Rush to the bathroom for a BM	35%	52%
Stiffen their legs or squeeze their bottom and legs together when they felt need to have a BM	45%	32%
Stain or soil underwear	58%	47%
Wet the bed	43%	29%
Become more active after passing a stool	53%	37%

Become less irritable after passing a stool	48%	45%
Mealtime and Dietary Behaviors - several times per month or more		
Turns their face or body away from food	68%	43%
Closes their mouth tightly when food is presented	57%	27%
Spits out food that they have put in their mouth	56%	34%
Stops eating after just a little food	66%	49%
Remains seated at the table until the meal at finished	45%	62%
Cries or screams during mealtimes	41%	18%
Is aggressive during mealtimes (hitting, kicking, scratching others)	25%	14%
Displays self-injurious behavior during mealtimes (hitting self, biting self)	12%	10%
Is disruptive during mealtimes (pushing/throwing utensils or food)	41%	18%
Is flexible about mealtime routines (e.g. times for meals, place settings, seating arrangements, meal locations)	74%	76%
Is willing to try new foods	35%	55%
Accepts or prefers a variety of foods	45%	62%
Prefers the same foods at each meal	89%	83%
Prefers food prepared in a particular way (e.g. eats mostly fried foods, cold cereals, raw vegetables)	78%	72%
Prefers to avoid eating a particular types of food group (e.g. vegetables, meats, dairy)	76%	71%
Strongly prefers certain types of food colors, textures, or temperatures	70%	73%
Refuses to eat foods that require a lot of chewing (e.g. eats only soft or pureed foods)	29%	19%
Prefers only sweet foods (e.g. candy, sugary cereals)	54%	47%
Is on a special diet (e.g. gluten free, casein free, FODMAPS, GAPS)	14%	15%
Drinks lots of water with meals	71%	69%
Other Behaviors		
Pushing on their own chest/neck/throat	6%	8%
Applying pressure to their abdomen by pushing on it or leaning on furniture	37%	28%

Unusual movements such as thrusting jaw, tilting head, arching back, or twisting neck/body	17%	18%
Frequent clearing of throat, swallowing, coughing, gagging, choking, or throat sounds wet or gurgly	25%	34%
Moaning or groaning for no apparent reason	18%	19%
Unexplained irritability, agitation, aggression, or screaming	53%	41%
Gritting teeth, wincing, or grimacing for no apparent reason	24%	22%
Biting themselves, putting their fist in their mouth, or hurting themselves in other ways	10%	16%
Avoid wearing tight clothing or clothing with waistbands	22%	25%
Chewing on shirts, eating non-edible objects	43%	39%
Pointing to stomach/tummy as if in pain	15%	15%
Direct vocalizations of pain (e.g. “tummy hurts” “stomach pain”)	26%	37%
Difficulty falling asleep or staying asleep	47%	53%

Table S2. Factor Loadings of ASD-GIRB Items in Seven-Factor Exploratory Factor Model

	Factor 1	**Factor 2**	**Factor 3**	**Factor 4**	**Factor 5**	**Factor 6**	**Factor 7**
	Constipation & Bowel Movement Pain	Doesn't want food at times	Particular with foods	Abdominal Pain / Vomiting / Gassy / Diarrhea	Incontinence / Soil / Wet bed	Aggressive/ Disruptive at Mealtimes	Other behaviors
Abdominal pain	0.03	0.01	-0.02	**0.83**	0.04	0.01	-0.05
Nausea, vomiting, or retching/dry heaving	-0.18	0.07	0.03	**0.51**	0.07	0.08	0.04
Bloating	**0.3**	-0.13	0.1	**0.37**	0.07	-0.04	0.17
Flatulence or gas	0.27	-0.06	0.09	**0.45**	0.11	0.04	-0.08
Diarrhea	-0.09	0.04	0.09	**0.33**	0.19	0.22	-0.10
Constipation	**0.58**	-0.13	0.04	0.16	0.17	-0.05	-0.01
Incontinence / Lack of voluntary control or urination or defecation	-0.1	0.0	-0.06	0.01	**0.78**	0.06	0.0
Fecal Retention / complete elimination of stool	**0.33**	0.0	0.06	0.03	**0.45**	-0.11	-0.18
Appear to feel pain when having a BM	**0.47**	0.03	-0.02	0.27	0.01	-0.06	0.04

Stiffen their legs or squeeze their bottom and legs together when they felt need to have a BM	**0.35**	0.20	0.03	-0.05	**0.38**	-0.11	0.24
Stain or soil underwear	0.03	0.11	0.04	0.05	**0.53**	0.0	0.12
Wet the bed	-0.06	-0.04	0.03	-0.03	**0.49**	0.18	0.06
Become more active after passing a stool	**0.68**	0.12	-0.02	-0.02	-0.02	0.05	0.11
Become less irritable after passing a stool	**0.79**	0.05	0.05	0.02	-0.09	0.13	-0.01
Turns their face or body away from food	0.05	**0.82**	0.03	0.02	-0.03	-0.02	-0.05
Closes their mouth tightly when food is presented	0.03	**0.54**	0.13	-0.11	0.04	0.18	0.0
Spits out food that they have put in their mouth	-0.06	**0.54**	0.06	0.10	0.09	0.04	0.11
Stops eating after just a little food	0.02	**0.57**	-0.03	0.06	0.06	-0.02	0.01
Remains seated at the table until the meal at finished	-0.01	**0.39**	0.03	-0.12	0.08	0.03	0.11
Cries or screams during mealtimes	-0.08	**0.35**	-0.06	0.08	-0.05	**0.36**	-0.01
Is aggressive during mealtimes (hitting, kicking, scratching others)	0.04	0.03	-0.04	0.05	-0.02	**0.8**	-0.03

Displays self-injurious behavior during mealtimes (hitting self, biting self)	0.05	-0.09	0.07	-0.08	0.06	**0.66**	0.13
Is disruptive during mealtimes (pushing/throwing utensils or food)	0.06	0.16	-0.02	0.01	0.06	**0.61**	-0.07
Is willing to try new foods	-0.19	0.14	**0.49**	0.08	-0.06	0.04	0.20
Accepts or prefers a variety of foods	-0.16	0.11	**0.5**	0.16	-0.01	-0.08	0.14
Prefers the same foods at each meal	-0.03	-0.03	**0.61**	0.03	0.06	0.07	-0.18
Prefers food prepared in a particular way (e.g. eats mostly fried foods, cold cereals, raw vegetables)	0.07	0.01	**0.58**	0.02	0.09	0.02	-0.18
Prefers to avoid eating a particular types of food group (e.g. vegetables, meats, dairy)	0.06	0.03	**0.75**	-0.11	-0.05	-0.06	0.03
Strongly prefers certain types of food colors, textures, or temperatures	0.07	0.0	**0.61**	0.03	-0.07	0.05	0.08
Applying pressure to their abdomen by pushing on it or leaning on furniture	0.18	0.0	0.02	0.26	0.05	0.04	**0.36**
Unusual movements such as thrusting jaw, tilting head, arching back, or twisting neck/body	0.11	-0.13	0.10	0.04	0.11	0.23	**0.35**

Gritting teeth, wincing, or grimacing for no apparent reason	0.11	0.05	-0.02	0.02	0.11	0.10	**0.51**
Biting themselves, putting their fist in their mouth, or hurting themselves in other ways	-0.02	-0.06	0.04	-0.05	0.15	**0.43**	0.22
Pointing to stomach/tummy as if in pain	0.03	0.02	0.04	**0.42**	-0.03	-0.05	0.24
Direct vocalizations of pain (e.g. “tummy hurts” “stomach pain”)	0.04	0.05	-0.02	**0.67**	-0.13	-0.05	0.06

*Note: Factor loadings greater or equal to 0.30 are **bolded**.*

Table S3. Correlations between ASD-GIRB Factors, among Children 6-17 Years

	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6	Factor 7
Factor 1	1	0.13	0.18	0.34	0.23	0.11	0.17
Factor 2	0.13	1	0.33	0.12	0.11	0.29	0.15
Factor 3	0.18	0.33	1	0.17	0.07	0.11	0.15
Factor 4	0.34	0.12	0.17	1	0.17	0.04	0.16
Factor 5	0.23	0.11	0.07	0.17	1	0.22	0.15
Factor 6	0.11	0.29	0.11	0.04	0.22	1	0.15
Factor 7	0.17	0.15	0.15	0.16	0.15	0.15	1

Factor 1 - Constipation & Bowel Movement Pain; Factor 2 - Doesn't want food at times; Factor 3 - Particular with foods; Factor 4 - Abdominal Pain / Vomiting / Gassy / Diarrhea; Factor 5 - Incontinence / Soil / Wet bed; Factor 6 - Aggressive/ Disruptive at Mealtimes; Factor 7 - Other behaviors

Table S4. Correlation between ASD-GIRB Factor Scores and CBCL Subscales, among Children 6-17 Years

	CBCL Subscales						
	Aggressive Behavior	**Withdrawn Depression**	**Rule Breaking Behavior**	**Somatic Complaints**	**Social Problems**	**Thought Problems**	**Attention Problems**
Factor 1 - Constipation & Bowel Movement Pain	0.11	0.16	0.04	**0.34**	0.27	0.23	0.20
Factor 2 - Doesn't want food at times	**0.31**	0.16	0.20	0.09	0.22	0.23	0.27
Factor 3 - Particular with foods	0.16	0.19	0.03	0.09	0.18	0.24	0.16
Factor 4 - Abdominal Pain / Vomiting / Gassy / Diarrhea	0.20	0.11	0.14	**0.54**	**0.35**	0.29	0.18
120Factor 5 - Incontinence / Soil / Wet bed	0.19	0.06	0.09	0.07	0.21	0.23	0.24
Factor 6 - Aggressive/ Disruptive at Mealtimes	**0.42**	0.18	0.21	0.10	0.18	**0.34**	0.25
Factor 7 - Other behaviors	0.27	0.17	0.03	0.25	0.24	**0.34**	0.28

*Moderate-sized correlations (r>=0.30) are **bolded**.*

Table S5. Mean Difference in ASD-GIRB Factor Scores by Functional Impairment

	Functional Impairment		
GI Tool Factor Scale Scores	**Missed School/Was Late**	**Missed Social/Family Activities**	**Trouble Falling/Staying Asleep**
Factor 1 - Constipation & Bowel Movement Pain	**3.92 vs.2.19**	**3.66 vs.2.2**	**2.99 vs.1.99**
Factor 2 - Doesn't want food at times	**2.8 vs.1.93**	**2.63 vs.1.92**	**2.42 vs.1.81**
Factor 3 - Particular with foods	4.14 vs.3.72	**4.25 vs.3.68**	**3.94 vs.3.64**
Factor 4 - Abdominal Pain / Vomiting / Gassy / Diarrhea	**3.72 vs.2.08**	**3.18 vs.2.16**	**3.04 vs.1.78**
Factor 5 - Incontinence / Soil / Wet bed	**2.08 vs.1.3**	**1.96 vs.1.29**	**1.72 vs.1.16**
Factor 6 - Aggressive/ Disruptive at Mealtimes	0.94 vs.0.72	1.02 vs.0.69	**1.02 vs.0.52**
Factor 7 - Other behaviors	**1.18 vs.0.59**	**0.93 vs.0.62**	**0.92 vs.0.46**

Table S5 shows the mean factor scale in children by presence versus absence of functional impairment. Bolded cells have a mean difference $p<0.05$ according to a t-test.

Autism Spectrum Disorder Gastrointestinal and Related Behaviors Inventory

Instructions for Parents/Primary Caregivers:
The purpose of this questionnaire is to assess the presence of gastrointestinal (GI) symptoms and related issues in children with autism spectrum disorder. This questionnaire may take up to 30 minutes to complete. The questionnaire should only be completed by the child's parent or another primary caregiver. There are no right or wrong answers to these questions, but please answer each question to the best of your ability. You may choose not to answer questions that make you feel uncomfortable.
Please note that all information will be kept strictly confidential.

Date questionnaire completed (DD/MM/YYYY)

_____

Please indicate your relationship to the child:

- ○ Mother
- ○ Father
- ○ Other primary caregiver

If Other, please describe:

_____

Please indicate your highest level of education completed.

- ○ Some high school
- ○ High school graduate or GED
- ○ Some college or associate degree education
- ○ College or associate degree
- ○ Graduate education

Please indicate your child's biological sex at birth.

- ○ Female
- ○ Male

Please indicate your child's gender identity.

- ○ Female
- ○ Male
- ○ Transgender
- ○ Non-binary, Gender-queer, or Gender-fluid
- ○ Other

Please indicate your child's race/ethnicity (select all that apply).

- ☐ American Indian or Alaska Native
- ☐ Asian
- ☐ Black or African American
- ☐ Hispanic or Latino
- ☐ Native Hawaiian or Other Pacific Islander
- ☐ White

Please indicate your child's current age in years.

_____

Has your child been diagnosed with any of the following conditions?

	No	Yes	Not Sure
Any gastrointestinal disorder			
Epilepsy/Seizure disorder			
Intellectual disability			
ADD/ADHD			
Sensory processing disorder			
Anxiety, panic, or phobia disorder			
OCD			
Tic/Tourette's disorder			
Depression			
Bipolar Disorder			
Sleep disorder			
Autoimmune disorder			
Allergies or Asthma			
Other			

If your child has been diagnosed with a gastrointestinal disorder, which gastrointestinal disorder(s) has your child been diagnosed with?

_____

_____

_____

_____

If other, please specify which other psychiatric/behavioral/medical condition(s) has your child been diagnosed with?

_____

_____

_____

_____

In the last 3 months, has your child experienced any of the following gastrointestinal symptoms?

	No	Yes	Not Sure
Abdominal Pain			
Nausea, Vomiting, or Retching/Dry Heaving			
Reflux or Heartburn			
Abdominal swelling or distension			
Bloating			
Flatulence or Gas			
Diarrhea			
Constipation			
Alternating constipation and diarrhea			
Incontinence / Lack of voluntary control over urination or defecation			
Fecal retention / incomplete elimination of stool			

How long has your child experienced these symptoms?

	Within the last 3 months only	3-5 months	6-11 months	1 year or longer	Not Sure	NA, has not experienced symptom in last 3 months
Abdominal pain						
Nausea, Vomiting, Retching/Dry Heaving						
Reflux or Heartburn						
Abdominal swelling or distension						
Bloating						
Flatulence or Gas						
Diarrhea						
Constipation						
Alternating constipation and diarrhea						
Incontinence / Lack of voluntary control over urination or defecation						
Fecal retention / incomplete elimination of stool						

In the last 3 months of your child having these symptoms...

	No	Yes	Not Sure	NA, has not experienced any symptoms in last 3 months
Do the symptoms get better after having a bowel movement (pooping)?				
Do the symptoms occur before eating or when hungry?				
Do the symptoms improve after your child eats?				
Do the symptoms worsen after your child eats?				
Has your child had trouble gaining weight?				

It can be difficult for parents/caregivers to accurately assess their child's pain level. How confident do you feel in your ability to assess your child's gastrointestinal pain?

- ○ Not confident at all
- ○ Slightly confident
- ○ Fairly confident
- ○ Completely confident

In the last 3 months, how often did your child usually have a bowel movement (BM), i.e. pooping?

- ○ Once a day
- ○ Less than once a day
- ○ More than once a day
- ○ Not sure

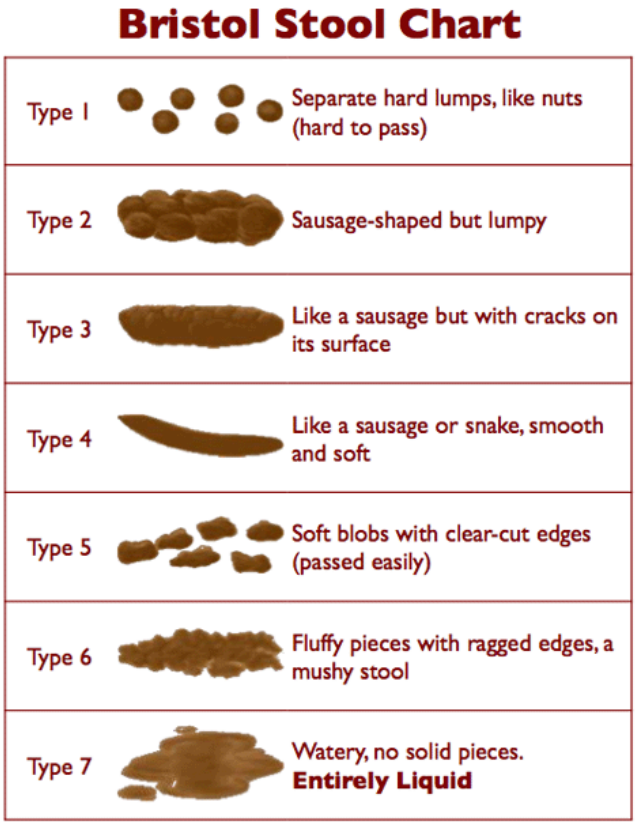
If you selected less than once a day, in the last 3 months, how often did your child usually have a bowel movement (BM), i.e. pooping?

- ○ 3-6 times per week
- ○ 1-2 times per week
- ○ Less than once per week
- ○ Not sure

If you selected more than once a day, in the last 3 months, how often did your child usually have a bowel movement (BM), i.e. pooping?

- ○ 2-3 times per day
- ○ 3+ times per day
- ○ Not sure

Bristol Stool Chart



In the last 3 months, what was your child's stool usually like? Of the 7 options below, please order them from most commonly like your child's stool to least commonly like your child's stool.
Write a 1 next to the most common stool, a 2 next to the second most common down to stool, etc. all the way to 7, the least common type of stool.

Please see the image above for reference.

_____ Type 1: Separate hard lumps

_____ Type 2: Lumpy and sausage like

_____ Type 3: A sausage shape with cracks in the surface

_____ Type 4: Like a smooth, soft sausage or snake

_____ Type 5: Soft blobs with clear-cut edges

_____ Type 6: Mushy consistency with ragged edges

_____ Type 7: Liquid consistency with no solid pieces

In the last 3 months, did your child...

	No	Yes	Not Sure
...Appear to feel pain when having a BM?			
...Rush to the bathroom for a BM?			
...Stiffen their legs or squeeze their bottom and legs together when they felt the need to have a BM?			
...Stain or soil underwear?			
...Wet the bed?			
...Become more active after passing a stool?			
...Become less irritable after passing a stool?			

Think about mealtimes with your child over the past 3 months. Rate the following items according to how often each occurs, using the following scale. My child...

	Never/Rarely	Several times per month	1-2 times per week	3 or more times per week	Not Sure
...Turns their face or body away from food					
...Closes their mouth tightly when food is presented					
...Spits out food that they have put in their mouth					
...Stops eating after just a little food					
...Remains seated at the table until the meal is finished					
...Cries or screams during mealtimes					
...Is aggressive during mealtimes (hitting, kicking, scratching others)					
...Displays self-injurious behavior during mealtimes (hitting self, biting self)					
...Is disruptive during mealtimes (pushing/throwing utensils or food)					
...Is flexible about mealtime routines (e.g. times for meals, place settings, seating arrangements, meal locations)					
...Willing to try new foods					
...Accepts/prefers variety of foods					

Think about mealtimes with your child over the past 3 months. Rate the following items according to how often each occurs, using the following scale. My child...

	Never/Rarely	Several times per month	1-2 times per week	3 or more times per week	Not Sure
...Prefers the same foods at each meal					
...Prefers food prepared in a particular way (e.g. eats mostly fried foods, cold cereals, raw vegetables)					
...Prefers to avoid eating a particular type of food group (e.g. vegetables, meats, dairy)					
...Strongly prefers certain types of food colors, textures, or temperatures					
...Refuses to eat foods that require a lot of chewing (e.g. eats only soft or pureed foods)					
...Prefers only sweet foods (e.g. candy, sugary cereals)					
...Is on a special diet (e.g. gluten free, casein free, FODMAPS, GAPS)					
...Drink lots of water with meals					

In the last 3 months, please indicate whether or not you've observed the following behaviors in your child?

	No	Yes	Not Sure
Pushing on their own chest/neck/throat			
Applying pressure to their abdomen by pushing on it or leaning on furniture			
Unusual movements such as thrusting jaw, tilting head, arching back, or twisting neck/body			
Frequent clearing of throat, swallowing, coughing, gagging, choking, or throat sounds wet or gurgly			
Moaning or groaning for no apparent reason			
Unexplained irritability, agitation, aggression, or screaming			
Gritting teeth, wincing, or grimacing for no obvious reason			
Biting themselves, putting their fist in their mouth, or hurting themselves in other ways			
Avoid wearing tight clothing or clothing with waistbands			
Chewing on shirts, eating non-edible objects			
Pointing to stomach/tummy as if in pain			
Direct verbalizations of pain (e.g. "tummy hurts" "stomach pain")			
Difficulty falling asleep or staying asleep			

Please select whether or not your child currently takes any of the following medications.

	No	Yes	Not Sure
Antidepressant			
Antipsychotic or Tranquilizer			
Anti-anxiety medication (e.g. benzodiazepine or hypnotics)			
Mood stabilizer			
Stimulant			
Anticonvulsant			
Prescription sleeping medication			
Hypotensive medication			
Other			

If other, please specify:

_____

In the last 3 months, have your child's GI symptoms led them to...

	Rarely/Never	Several times per month	1-2 times per week	3 or more times per week	Not Sure
Get to school late, miss school, or leave school early (including missing parts of class)?					
Miss social or family activities?					
Have trouble falling asleep or staying asleep?					

This GI questionnaire was designed using items from the Autism Treatment Network GI Inventory and the Brief Autism Mealtime Behaviors Inventory (BAMBI). This Network activity was supported by Autism Speaks and cooperative agreement UA3 MC11054 through the U.S. Department of Health and Human Services, Health Resources and Services Administration, Maternal and Child Health Research Program to the Massachusetts General Hospital. This work was conducted through the Autism Speaks Autism Treatment Network. This work was funded by the Wendy Klag Center for Autism and Developmental Disabilities.

CHAPTER 5: INTERACTION BETWEEN MATERNAL IMMUNE ACTIVATION AND ANTIBIOTIC USE DURING PREGNANCY AND RISK OF AUTISM SPECTRUM DISORDER IN THE CHILD

Coauthors: Martha Brucato[1,2,3], Xiumei Hong[4], Noel Mueller[2], M. Daniele Fallin[3,5,+], Xiaobin Wang[4,6,+]

[+]These authors contributed equally.

[1]Johns Hopkins University School of Medicine; [2]Department of Epidemiology; [3]Wendy Klag Center for Autism and Developmental Disabilities, Johns Hopkins Bloomberg School of Public Health;[4]Department of Population, Family and Reproductive Health, Johns Hopkins Bloomberg School of Public Health and The Center on the Early Life Origins of Disease, Johns Hopkins Bloomberg School of Public Health; [5] Department of Mental Health, Johns Hopkins Bloomberg School of Public Health; [6] Department of Pediatrics, Johns Hopkins University School of Medicine

5.1 Background

Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder characterized by two core domains of symptoms: social communication and impairment, and restricted, repetitive behaviors, interests, or activities[1]. The prenatal period and early-life period are considered to be the critical windows for the development of ASD, given the brain's susceptibility to environmental stressors[2–6].

Prenatal exposure to maternal immune activation (MIA) has been implicated as a risk factor for the development of neuropsychiatric disorders, and in particular, schizophrenia and ASD[7–9]. Animal studies have demonstrated that maternal immune activation leads to behavioral abnormalities such as decreased social approach and ultrasonic vocalizations, and increased repetitive grooming and marble burying behavior in the offspring[10–14]. These behavioral changes are paralleled by alterations in the immune profiles of offspring, in particular levels of IL-6 and IL-17α[13,15–17].

Human studies have also found a strong link between prenatal exposure to MIA and risk of ASD in the offspring. Overall, bacterial [18–20], viral [19,20], and genitourinary infections[18,21], as well as fever[21–23] have been implicated as risk factors for ASD. However, there is substantial heterogeneity in the findings across these studies. A meta-analysis and systematic review by Jiang et al. found that overall, maternal infections, particularly those requiring hospitalization, increase the risk for ASD[24]. Any maternal infection in the second trimester in particular, bacterial infections overall and in particular during the second and third trimesters, and viral infections over the entire pregnancy (but not within a particular trimester) were all significantly

associated with higher risk of ASD[24]. Despite the overwhelming evidence that an activated immune system in the pregnant mother increases the risk for neurodevelopmental abnormalities in the child, the conditions under which this elevated risk occurs are unclear.

Human gut microbiota, or the microbes living in our gastrointestinal tract, and their genetic material (the gut microbiome) have emerged as key players in health and disease. The gut microbiome is malleable across the life course, which makes it an attractive target for intervention[25]. Until recently, the infant gut microbiome was thought to be sterile until birth, when the infant is inoculated with the mothers vaginal (vaginal delivery) or skin microbiome (C-section)[26]. However, studies now show that bacteria can be detected in fetal membranes, amniotic fluid, and placenta, although this finding is not without controversy[27–33]. Early immunological and metabolic programming by the gut microbiome may have long-term consequences for human health[34–40]. Further, breast milk microbiota, which is critical to an infant's development, is influenced by both delivery mode and intrapartum antibiotic exposure[41–44]. Given that the development of the infant gut microbiome parallels critical windows of neurodevelopment, and that the microbiome is particularly susceptible to environmental influences during pregnancy and early life, the infant gut microbiome represents a potential opportunity for better understanding neurodevelopment and potentially preventing or treating neuropsychiatric disorders, including ASD[45,46].

Very recent animal studies indicate that the maternal gut microbiome plays a critical role in regulating the maternal immune response and subsequent neurodevelopment of the offspring[47], specifically that among pregnant dams exposed to immune activation, antibiotic use protects

against the MIA-associated neurodevelopmental abnormalities seen in the offspring. The aim of this study was to assess whether antibiotic use during pregnancy modifies the association between prenatal exposure to maternal immune activation and subsequent risk of autism spectrum disorder, in a prospective, enriched-risk cohort, the Boston Birth Cohort (BBC) study. We hypothesized that antibiotic use would be protective against ASD in the presence of maternal immune activation.

5.2 Methods

5.2.1. Sample

Our sample consisted of mother-child pairs from the Boston Birth Cohort (BBC), a prospective birth cohort recruited from the Boston Medical Center[48,49]. Initiated in 1998, the BBC was designed to investigate environmental and genetic determinants for preterm delivery. Because it is enriched for preterm deliveries, it is a useful cohort for studying autism epidemiology, given that preterm delivery is a risk factor for the development of ASD[50]. The BBC has been described in detail elsewhere [23,48], but briefly: Women with a live, singleton birth at Boston Medical Center are eligible for recruitment. Pregnancies involving IVF, chromosomal abnormalities, major birth defects, and preterm deliveries due to maternal trauma are excluded. Eligible participants are contacted 24-72 hours following birth for consent and study enrollment. Children who seek postnatal care at Boston Medical Center are able to be followed up for developmental outcomes, such as ASD status. Most pregnant women in the Boston Medical Center are urban, low-income minority individuals who receive healthcare through public assistance based insurance programs[23,48,49].

5.2.2. Outcome Classification

Electronic medical record ICD-9 CM diagnostic codes from inpatient, outpatient, and emergency department visits at the Boston Medical Center (October 1, 2003 through September 31, 2015) were used to classify children as ASD cases or non-ASD controls. Children were classified as having ASD if their medical recordings contained any of the ICD-9 CM codes at least once: 299.00, 299.01, 299.80, 299.81, 299.90, or 299.91[23]. Children were classified as non-ASD controls if they had none of the above ICD-9 CM codes.

5.2.3. Definition of maternal immune activation

The details of defining maternal immune activation have previously been described elsewhere[23]. Briefly, maternal immune activation was defined as any of the following exposures: prenatal flu, prenatal fever (excluding intrapartum), prenatal genitourinary (GU) tract infections, and intrapartum (labor and delivery) fever. A standardized postpartum questionnaire was administered to the mother 24-72 hours following delivery for self-report of flu, fever, and GU infection. Intrapartum fever was defined as a >38° C temperature in the mother and abstracted from medical charts during labor and delivery. For flu and fever, overall as well as trimester-specific variables were used, and an overall variable only was used for genitourinary infection. All individuals were dichotomized as "exposed" or "unexposed" for these four MIA variables. Lastly, a combined MIA variable was created; women were classified as being exposed to MIA if they were exposed to flu, fever, GU infection, or intrapartum fever at any point while pregnant.

5.2.4. Definition of antibiotic use during pregnancy

Use of an antibiotic during pregnancy/labor and delivery was derived from three sources of information. First, in the standardized postpartum questionnaire, mothers were asked 'What medicines did you take during your pregnancy excluding vitamins?'. Women could list up to 5 medications and indicate which trimester the medication was taken. Second, inpatient and outpatient antibiotic treatments during the entire pregnancy were extracted from electronic health records (EHR). Lastly, use of intrapartum antibiotics was extracted from medical charts during labor and delivery. We combined these three sources of information to obtain one aggregate, binary variable representing whether or not a woman was exposed to an antibiotic during pregnancy or the labor and delivery period. Women who did not have EHR antibiotic information, who did not report antibiotic use on the standardized questionnaire, and who did not receive an intrapartum antibiotic were classified as not exposed to antibiotics during pregnancy. We were not able to separate antibiotic use by the type of antibiotic or by trimester because of sparse data. For a list of which antibiotics were extracted from the postpartum maternal questionnaire and the EHR records, see Supplemental Table 10.

5.2.5. Covariate definitions

We controlled for the following covariates in our analysis: demographic characteristics of the mother (maternal education, marital status, age at delivery), clinical or health characteristics of the mother or having to do with the pregnancy (BMI, diabetes or gestational diabetes, preeclampsia, preterm delivery, mode of delivery). Maternal education and marital status were obtained from the postpartum maternal questionnaire. Child sex, preterm status, and C-section

delivery was obtained from medical charts. Maternal BMI, diabetes/gestational diabetes, and preeclampsia were obtained from the postpartum maternal questionnaire.

We defined mothers' educational attainment as a binary variable (high school graduation/GED or lower versus some college or higher). Marital status was dichotomized to "married" or "not married", with not married including women who were single, divorced, separated, or widowed. BMI was categorized as "not overweight" (BMI <25), "overweight" (BMI 25-29.9), or "obese" (BMI >29.9) Race was self-reported by choosing one of the nine categories: Black/African American (Black, African American, Haitian, Cape Verdean, Caribbean), Asian (Asian and Pacific Islander), White, Hispanic, mixed race, and all others. However, race was not included as a covariate in our regression models because of sparseness across racial categories.

5.2.6. Analytic dataset

Out of 3,123 mother-child pairs with non-missing ASD information, 28 were removed for missing information on child sex (n=15), delivery type (n=28), or preterm status (n=15), leaving 3,095 pairs (n=142 ASD, n=2,953 non-ASD). Complete-cases analyses were used for regression models.

5.2.7. Statistical analyses

All data cleaning and analyses were performed in R Studio version 1.1.383 (R version 3.4.3). Descriptive characteristics of the analytic sample were carried out, stratified by ASD and antibiotic use. We first carried out unadjusted and adjusted logistic regression models examining the main effect of each of 11 MIA exposures (binary MIA reflecting any infection during

pregnancy and birth, flu overall and in each trimester, fever overall and in each trimester, GU infection overall, and intrapartum fever) with ASD as the outcome. Then, we conducted a series of 11 logistic regression models with each MIA exposure, as a predictor, antibiotic use as an interaction term, and ASD diagnosis as the outcome. We ran both unadjusted models and models adjusted for child sex, maternal education, marital status, maternal age, maternal BMI, diabetes/gestational diabetes, preeclampsia, and C-section. Models with an interaction term p-value < 0.10 were suggestive of possible interaction, and were followed up with stratified analyses. These models estimated the association between MIA and odds of ASD, stratified by antibiotic use.

We carried out a series of 5 stratified analysis models: Model 1 was unadjusted. Model 2 included child sex, maternal education, marital status, maternal age, maternal BMI, diabetes/gestational diabetes, and preeclampsia. Model 3 included model 2 covariates as well as preterm status. Model 4 included model 2 covariates as well as C-section delivery. Model 5 included model 2 covariates and both preterm status and C-section delivery.

Lastly, we carried out some sensitivity analyses. First, we repeated our regression modeling excluding children less than three years old, and again, less than two years old, to account for the possibility that ASD diagnosis would be much less likely before the age of three. We also excluded women who did not have EHR antibiotic information, and who did not report antibiotic use on the standardized questionnaire, and who did not receive an intrapartum antibiotic.

5.3 Results

5.3.1. Sample characteristics

Out of 3,095 children, 142 (4.6%) had a diagnosis of ASD, 79% of mothers used an antibiotic during pregnancy or in labor and delivery and over half (54%) experienced maternal immune activation. Over 90% of our study population was of non-white race. The most common type of maternal immune activation was genitourinary infection, followed by flu and then fever. Children with ASD were significantly more likely to be male, younger in age, have an older mother, have a mother with obesity or diabetes/gestational diabetes, and be born preterm (Table 1). Children with maternal exposure to antibiotics were more likely to have a mother with diabetes/gestational diabetes, and be born preterm or by C-section. The frequencies of maternal immune activation exposures, stratified by ASD status and by antibiotic use during pregnancy, are shown in Table 2. In these crude estimates, the only significant association was between having intrapartum fever and intrapartum antibiotics.

Table 1. Descriptive Characteristics of Total Sample, Stratified by ASD and Antibiotic Use in Pregnancy (% or mean (SD))

	Total Sample (n=3,095)	ASD (n=142)	No ASD (n=2,953)	P-value[a]	Antibiotic during Pregnancy (n=2,386)	No Antibiotic during Pregnancy (n=709)	P-value[a]
ASD	5%	100%	0%	---	5%	3%	0.05
Antibiotic Use during Pregnancy	79%	86%	79%	0.05	100%	0%	---
Male child	51%	75%	49%	<0.0001	51%	49%	0.36
Child age	8.1 (3.7)	7.5 (3.8)	8.1 (3.7)	<0.0001	6.9 (2.9)	11.2 (3.6)	
Maternal age	28.5 (6.5)	30.0 (6.2)	28.5 (6.5)	<0.0001	28.1 (6.4)	28.5 (6.6)	
Maternal education: high school grad or below	64%	58%	65%	0.11	64%	66%	0.32
Mother not married	67%	64%	67%	0.59	67%	66%	0.69
Maternal race: Black, Hispanic, Asian, Mixed, Other (not white)	93%	96%	93%	0.20	92%	94%	0.41
Maternal BMI							
Not overweight	49%	43%	49%		49%	52%	
Overweight	27%	24%	27%	0.04	27%	28%	0.11
Obese	24%	33%	23%		25%	20%	
Diabetes/Gestational Diabetes	11%	18%	11%	0.02	13%	7%	<0.001
Preeclampsia	11%	9%	12%	0.34	12%	10%	0.24
Maternal Immune Activation (combined)	54%	51%	54%	0.52	54%	52%	0.57
Preterm	29%	37%	29%	0.03	31%	21%	<0.0001
C-section	36%	40%	36%	0.34	39%	26%	<0.0001

[a]P-value for Chi-square or T-test difference between strata (ASD vs. no ASD and Antibiotic vs. No Antibiotic during Pregnancy)

Table 2. Frequency of maternal immune activation, stratified by ASD and Antibiotic Use in Pregnancy (%)

Maternal Immune Activation Exposures	ASD (n=142)	No ASD (n=2,953)	P-value[a]	Antibiotic during Pregnancy (n=2,386)	No Antibiotic during Pregnancy (n=709)	P-value[a]
Maternal immune activation (any)	51%	54%	0.52	54%	52%	0.57
Intrapartum fever	7%	6%	0.88	7%	3%	<0.001
Genitourinary infection	28%	35%	0.16	35%	32%	0.10
Flu overall pregnancy	20%	18%	0.72	17%	21%	0.04
Flu Trimester 1	5%	5%	0.97	5%	6%	0.20
Flu Trimester 2	10%	8%	0.44	8%	8%	0.55
Flu Trimester 3	8%	8%	1.00	7%	10%	0.01
Fever overall pregnancy	12%	11%	0.64	11%	10%	0.77
Fever trimester 1	3%	4%	0.94	3%	4%	0.13
Fever trimester 2	5%	4%	0.60	4%	3%	0.40
Fever trimester 3	5%	4%	0.81	4%	3%	0.72

[a]P-value for Chi-square or T-test difference between strata (ASD vs. no ASD and Antibiotic vs. No Antibiotic during Pregnancy)

5.3.2. Main effect of maternal immune activation on odds of ASD

In both unadjusted and adjusted analyses there were no significant associations between any MIA exposure and ASD as the outcome (Table 3). We chose to include C-section as a covariate in these main effect models, since they were included as a covariate in our interaction models. However, we also repeated the models without adjusting for C-section, given its potential role as a mediator of the association between maternal immune activation and ASD. Removal of this variable did not change the magnitude, significance, or inference of our findings.

Table 3. Regression Models Estimating Association between Maternal Immune Activation Exposures and Odds of ASD

Maternal Immune Activation Exposure	Unadjusted Odds Ratio (95% Confidence Interval)	Adjusted Odds Ratio[b] (95% Confidence Interval)
Flu Overall Pregnancy	1.11 (0.70 , 1.70)	1.30 (0.80, 2.03)
Flu, Trimester 1	0.90 (0.35 , 1.92)	1.05 (0.40 , 2.27)
Flu, Trimester 2	1.32 (0.70 , 2.30)	1.34 (0.68 , 2.4)
Flu, Trimester 3	0.97 (0.47 , 1.78)	1.21 (0.58 , 2.26)
Fever Overall Pregnancy	1.18 (0.67 , 1.97)	1.39 (0.77 , 2.35)
Fever, Trimester 1	0.85 (0.26 , 2.07)	1.02 (0.31 , 2.54)
Fever, Trimester 2	1.35 (0.56 , 2.76)	1.40 (0.57 , 2.93)
Fever, Trimester 3	1.23 (0.47 , 2.63)	1.48 (0.56 , 3.24)
Genitourinary Infection Overall Pregnancy	0.74 (0.50 , 1.09)	0.78 (0.51 , 1.16)
Intrapartum fever	1.13 (0.52 , 2.14)	0.93 (0.35 , 2.0)
Maternal Immune Activation (any)	0.87 (0.60 , 1.26)	0.96 (0.65 , 1.44)

[b]Models are adjusted for child sex, maternal education, marital status, maternal age, maternal BMI, diabetes/gestational diabetes, preeclampsia, and C-section delivery

5.3.3. Interaction between maternal immune activation and antibiotic use

In unadjusted analyses, there were no significant interactions at the $p<0.10$ level (Supplemental Tables 1-9). After adjusting for child sex, maternal education, marital status, maternal age, maternal BMI, diabetes/gestational diabetes, and preeclampsia, there was suggestive evidence of an interaction between flu in the second trimester and antibiotic use on odds of ASD ($p<0.10$). No other regression models showed evidence of any significant interaction (Supplemental Tables 1-9).

We further examined the association between flu and ASD risk, stratified by antibiotic use in pregnancy or labor and delivery. Among women who received an antibiotic, there was no significant association between flu in trimester two and odds of ASD, in either unadjusted or adjusted models (Figure 1). In this subgroup, women with a high school diploma/GED or less, relative to women with some college experience, were significantly less likely to have a child with ASD (OR=0.61, 95% CI 0.40-0.95), while mothers with a male child (OR=2.89, 95% CI 1.81-4.79) or a preterm birth (OR =1.72, 95% CI 1.09-2.68) were more likely to have a child with ASD ($p<0.05$) (Table 4 and Figure 1).

However, among women who did not receive an antibiotic during pregnancy, flu in the second trimester significantly increased the odds of ASD in the child after adjusting for potential confounders. In this subgroup of women, flu in the second trimester was associated with a 4.43 fold increase in the odds of ASD (95% CI 1.13-14.69), adjusting for demographic and clinical characteristics, including preterm birth and C-section delivery, although the confidence interval was wide, reflecting the small sample size in this strata. Across these models, child male sex

(OR=3.67, 95% CI 1.24-13.48) and maternal education at or below high school diploma/GED (OR=4.78, 95% CI 1.28-31.35) were both associated with significantly greater odds of ASD (Table 4 and Figure 1).

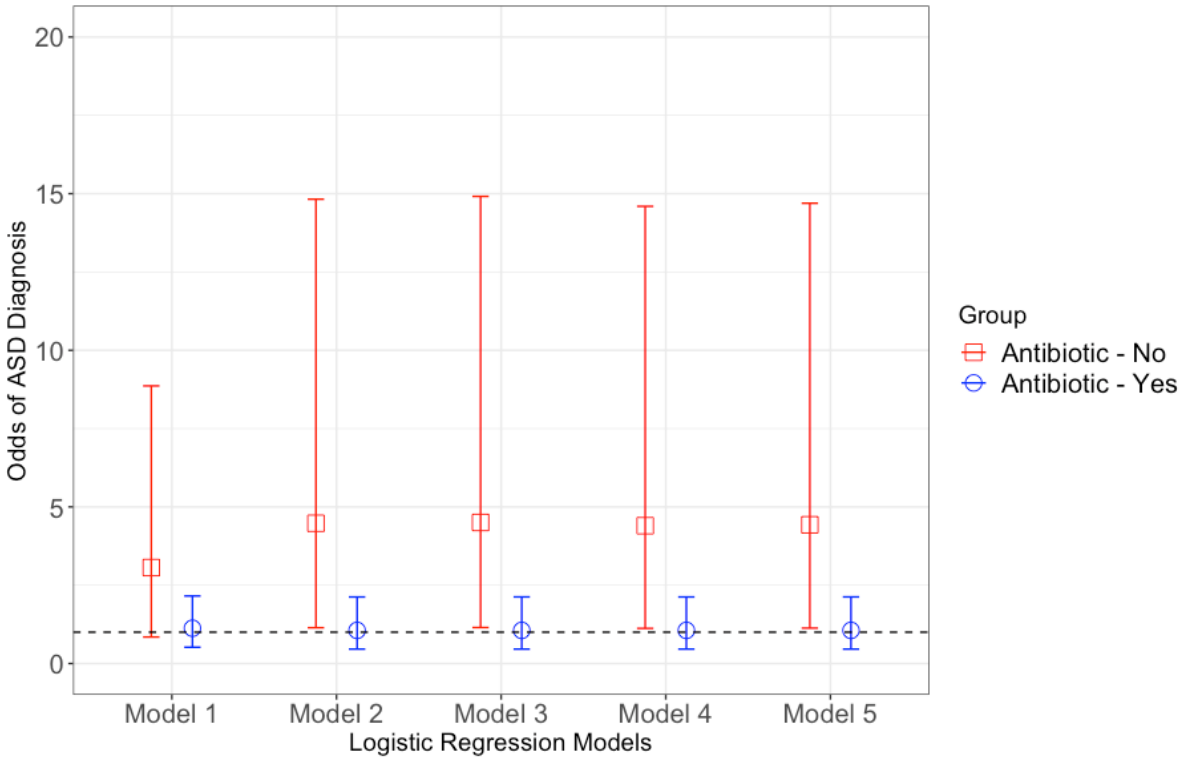
The magnitude, significance, and interpretation of our results did not differ when restricting to children >=2 years old or to children >= 3 years old (data not shown). Further, our inference did not change after excluding women with missing EHR antibiotic information, though some of the models were not estimable given the smaller sample size of our sample (data not shown).

Table 4. Regression Models Estimating Association between Flu in Second Trimester and Odds of ASD, Stratified by Antibiotic Use during Pregnancy

	Odds Ratio (95% Confidence Interval)	
Model	**Flu Trim 2, among Women who did use Antibiotic during Pregnancy**	**Flu Trim 2, among Women who did not use Antibiotic during Pregnancy**
Model 1	1.13 (0.52 , 2.16)	3.06 (0.85 , 8.86)
Model 2	1.06 (0.46 , 2.12)	4.48 (1.14 , 14.82)*
Model 3	1.06 (0.46 , 2.13)	4.50 (1.15 , 14.91)*
Model 4	1.06 (0.46 , 2.12)	4.40 (1.12 , 14.60)*
Model 5	1.06 (0.46 , 2.13)	4.43 (1.13 , 14.69)*

Model 1 was unadjusted. Model 2 included child sex, maternal education, marital status, maternal age, maternal BMI, diabetes/gestational diabetes, and preeclampsia. Model 3 included model 2 covariates as well as preterm status. Model 4 included model 2 covariates as well as C-section delivery. Model 5 included model 2 covariates and both preterm status and C-section delivery. *P-value for estimates is <0.05.

Figure 1. Odds Ratio Estimates and Confidence Intervals for Association between Flu in Second Trimester and ASD, Stratified by Antibiotic Use during Pregnancy and Adjusting for Series of Covariates



Model 1 was unadjusted. Model 2 included child sex, maternal education, marital status, maternal age, maternal BMI, diabetes/gestational diabetes, and preeclampsia. Model 3 included model 2 covariates as well as preterm status. Model 4 included model 2 covariates as well as C-section delivery. Model 5 included model 2 covariates and both preterm status and C-section delivery. *P-value for estimates is <0.05.

5.4 Discussion

We examined whether the prospective relationship between maternal immune activation on risk for ASD is modified by antibiotic use in pregnancy, in a predominantly urban minority population. Our results suggest that flu during the second trimester increases the risk for ASD in the offspring, among women who are not exposed to antibiotics during pregnancy. In other words, among women who have flu in their second trimester, exposure to an antibiotic during pregnancy appears to protect against the increased risk for ASD in the child. Our finding was specific to second trimester, and was not replicated in first or third trimester, or in overall pregnancy. We also did not find any evidence of significant interactions between other non-flu MIA exposures and antibiotic use.

To our knowledge, this is the first study in humans to test for interaction between maternal immune activation and antibiotic use in pregnancy on ASD risk. However, this study follows a number of papers examining antibiotic use or treatment of infection on ASD. Fever-associated risk for ASD or developmental disabilities has been found to be decreased among mothers who took antipyretic medications during pregnancy, implying that treatment for the maternal immune activation was protective[21]. The literature regarding treatment with antibiotic use in particular on ASD risk has been mixed. A 2012 study of the Danish National Birth Cohort found a small but elevated risk of ASD and infantile autism among women who self-reported taking an antibiotic in pregnancy, though the study did not disentangle whether the association was causal or due to confounding by indication[51]. A 2018 study using Danish registry data found that infections since birth treated with anti-infective agents were associated with increased of being diagnosed with a mental disorder or redeeming a prescription for psychotropic medication. Antibiotic use in

particular was associated with increased risk for a mental disorder[9]. A study out of the Autism Treatment Network similarly found that antibiotic use and ear infections were associated with increased risk for ASD, and the antibiotic in particular seemed to account for the association between ear infection and ASD[52]. However, another recent study also using Danish registry data aimed to assess whether otitis media, previously associated with risk for ASD[53], was itself associated with ASD or whether antibiotic treatment for otitis media was the true risk factor[54]. The authors found that exposure to exposure to otitis media and antibiotic use were both independently associated with increased risk for ASD. Otitis media remained a significantly associated with ASD even after controlling for exposure to antibiotics. There was no evidence of any interaction between otitis media and antibiotic use on ASD, given that the risk for ASD among children who were exposed to both otitis media and antibiotics did not change meaningfully from the risk among children only exposed to antibiotics[54]. Importantly, these studies differ from the present study in that they examined infections and antibiotics during early-life, but not during pregnancy.

The findings from our study support recent animal literature demonstrating that antibiotics mitigate the maternal immune activation insult on the risk for ASD in the developing offspring, and suggest that the maternal gut microbiome may play a critical role in modulating the immune system's response on the developing brain[47]. Specifically, MIA-associated phenotypes (increased repetitive behaviors, anxiety, social interaction deficits, as well as cortical patches) require maternal gut bacteria that promote differentiation of Th17 cells, for secretion of Il-17a. Treatment of pregnant dams with vancomycin, a broad-spectrum antibiotic prevented the neurodevelopmental abnormalities associated with MIA by decreasing the amount of Th17 cells

in the small intestine, thus decreasing levels of IL-17a circulating in the maternal plasma. Critically, transplantation of the mouse intestinal microbiome with commensal bacteria from humans previously found to induce Th17 cells also led to the neurodevelopmental abnormalities[47]. In sum, this demonstrates that the composition of the maternal gut microbiome, as well as factors that influence it such as antibiotics, seem to determine whether exposure to maternal immune activation leads to increased risk of neurodevelopmental abnormalities in the offspring. Notably, animal research has shown that the influence of MIA on the offspring is modified by the genetic strain of the mice[10,55], and that strains differ in their immune and microbiota profiles, in a sex-dependent manner[56].

In this study, we found that flu in the second trimester increased risk for ASD in the child, among women who did not receive any antibiotic during pregnancy or labor and delivery. Viral infections during pregnancy have previously been implicated with increased for ASD, though findings have not been specific to a particular trimester, and bacterial infections during second and third trimester in particular, have been associated with increased ASD risk[18–20,24]. One possibility is that women who self-reported having the flu during pregnancy may not have truly had influenza, but might have had a different infection, perhaps of bacterial nature. We also cannot rule out that the possibility that other trimester-specific flu/fever exposures would have been implicated had enough observations been present. Our interaction models included between 4-61 individuals with both ASD and some MIA exposure, and these individuals were further stratified by antibiotic use. Among this group, exposure to an antibiotic was more common. Among those that did not receive an antibiotic, women who had flu in second trimester made up the largest group (n=4), compared to the other trimester-specific flu/fever

variables. As evidenced in Tables 3a and 3b and Figure 1, we had very wide confidence intervals for our estimates, and some of our models were not estimable. Therefore, we may have been underpowered to detect effects from other trimester-specific exposures. Because this study was enriched for preterm births, infections in third trimester are less likely. Further, women may not recall first-trimester infections or may not have known they were pregnant during that time. For these reasons, our second-trimester finding may not necessarily reflect a true critical window compared to other trimesters. Prior literature stresses the importance of considering ASD risks in the context of specific infectious agents as well as the timing in pregnancy. It's possible that we did not find any combined effect of flu, fever, genitourinary infection, or any MIA for these reasons.

This study should be interpreted in the context of several limitations. First, our sample size was quite small when looking at the interaction of MIA with antibiotic use on ASD status. Our effect estimates were not precise, and we were underpowered. We used a complete-case analysis, which has the potential to introduce biases. However, the ASD outcome was not associated with complete case status after adjusting for our covariates[57]. Future studies in larger cohorts are needed to replicate our findings. Second, we relied on self-report information on infections. Therefore, our data may be susceptible to recall errors and misclassification. However, we do not think recall bias by ASD status is likely, since women recalled their infectious exposures just after the birth of their child, prior to ASD diagnosis. For women who did not have antibiotic information in EHR (because only women with an antibiotic prescription are included in EHR), we assumed that these women were not exposed to antibiotics, provided that they also did not self-report antibiotic use on the standardized questionnaire and did not receive intrapartum

antibiotic. However, our sensitivity analyses revealed similar inferences when excluding these women. Next, if a pregnant woman was prescribed an antibiotic outside of Boston Medical Center and did not self-report this in the postpartum maternal questionnaire, she would be incorrectly classified as not being exposed to antibiotics during her pregnancy. Assuming this missingness is not differential with respect to ASD, our inference would not be affected. However, if the likelihood of being incorrectly classified as being unexposed were related to ASD, then our strata-specific associations would be more similar to each other, meaning we would have less evidence of an interaction effect.

An important consideration is that this study did not look at the effect of treatment of an infection with an antibiotic on ASD risk. Rather, antibiotics could have been prescribed for any reason. Because we were interested in the effect of antibiotics at any point in pregnant on the maternal gut microbiota, this study does not suggest that treatment of infections with an antibiotic completely negates the risk of ASD on the offspring. Rather, it suggests that the effect of the antibiotic on the mother and on the intrauterine environment potentially modifies the influence of MIA on the child. While we adjusted for a number of potential confounders (demographic and clinical variables, and in particular preterm status and C-section delivery), it is still possible that antibiotic use was a proxy for the condition it was initially prescribed for.

The findings from this study do not suggest that antibiotics should be unnecessarily prescribed or are by themselves protective against ASD. Indeed, antibiotics also carry risks to the mother and developing fetus, and prior literature has shown that antibiotics themselves might increase the risk of ASD[9,51–53,58–60].

Our study had a number of strengths, however. First, we assessed for the first time the interaction between maternal immune activation and antibiotic use on ASD. Within a prospective, enriched-risk birth cohort, we demonstrated that the association between maternal immune activation is modified by the use of antibiotics during pregnancy. Antibiotic use and ASD were both assessed by electronic health record data, and we relied on self-report of infections, slightly after pregnancy, which is more sensitive than use of electronic health record data, which only capture more serious infections. The interaction effect we found was maintained after adjusting for demographic factors (child sex, maternal education, marital status and age) as well as clinical variables (maternal BMI, diabetes/gestational diabetes, preeclampsia, preterm status, C-section delivery). The findings from this study are in line with recent animal literature showing that antibiotic use affects the maternal gut microbiome, modifying the influence of maternal immune activation on neurodevelopment and autism-like symptoms in the offspring. The maternal gut microbiome may very well be the missing link in understanding why maternal exposures, including immune activation, have heterogeneous effects on the neurodevelopment of children.

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Table S1. Interaction between Flu in Overall Pregnancy and Antibiotic Use during Pregnancy on Odds of ASD

	OR (95% CI)	P-value	OR (95% CI)	P-value
Flu Overall Pregnancy	1.77 (0.61 , 4.57)	0.26	2.12 (0.71 , 5.77)	0.15
Antibiotic during Pregnancy	1.78 (1.02 , 3.36)	0.06	1.67 (0.91 , 3.37)	0.12
Child sex: male	---		3.04 (1.97 , 4.82)	0.00
Maternal education: HS graduation or below	---		0.79 (0.53 , 1.19)	0.25
Marital status: nor married	---		1.05 (0.69 , 1.62)	0.83
Maternal age (years)	---		1.03 (1.0 , 1.07)	0.04
Maternal BMI (ref: not overweight)	---			
Overweight	---		1.15 (0.70 , 1.86)	0.58
Obese	---		1.55 (0.96 , 2.50)	0.07
Diabetes/Gestational Diabetes	---		1.54 (0.89 , 2.54)	0.11
Pre-eclampsia	---		0.70 (0.34 , 1.32)	0.31
Flu Overall Preg*Antibiotic during Pregnancy	0.59 (0.20 , 1.89)	0.35	0.55 (0.18 , 1.83)	0.31

Table S2. Interaction between Flu in Trimester 1 and Antibiotic Use during Pregnancy on Odds of ASD

	OR (95% CI)	P-value	OR (95% CI)	P-value
Flu Trim 1	0.86 (0.05 , 4.38)	0.89	0.86 (0.05 , 4.49)	0.89
Antibiotic during Pregnancy	1.54 (0.95 , 2.65)	0.10	1.38 (0.82 , 2.47)	0.25
Child sex: male	---		3.04 (1.98 , 4.83)	0.00
Maternal education: HS graduation or below	---		0.80 (0.53 , 1.19)	0.27
Marital status: nor married	---		1.03 (0.68 , 1.59)	0.88
Maternal age (years)	---		1.03 (1.0 , 1.07)	0.04
Maternal BMI (ref: not overweight)	---			
Overweight	---		1.15 (0.70 , 1.86)	0.58
Obese	---		1.55 (0.96 , 2.50)	0.07
Diabetes/Gestational Diabetes	---		1.52 (0.88 , 2.51)	0.12
Pre-eclampsia	---		0.70 (0.33 , 1.31)	0.29
Flu Trim 1*Antibiotic during Pregnancy	0.92 (0.12 , 18.9)	0.94	1.09 (0.14 , 22.72)	0.94

Table S3. Interaction between Flu in Trimester 2 and Antibiotic Use during Pregnancy on Odds of ASD

	OR (95% CI)	P-value	OR (95% CI)	P-value
Flu Trim 2	3.06 (0.85 , 8.86)	0.06	3.56 (0.95 , 10.81)	0.04
Antibiotic during Pregnancy	1.78 (1.06 , 3.21)	0.04	1.66 (0.95 , 3.16)	0.09
Child sex: male	---		3.03 (1.97 , 4.80)	0.00
Maternal education: HS graduation or below	---		0.80 (0.54 , 1.20)	0.27
Marital status: nor married	---		1.05 (0.69 , 1.63)	0.81
Maternal age (years)	---		1.03 (1.0 , 1.07)	0.04
Maternal BMI (ref: not overweight)	---			
Overweight	---		1.15 (0.70 , 1.86)	0.59
Obese	---		1.55 (0.96 , 2.50)	0.07
Diabetes/Gestational Diabetes	---		1.52 (0.88 , 2.51)	0.11
Pre-eclampsia	---		0.69 (0.33 , 1.30)	0.29
Flu Trim 2*Antibiotic during Pregnancy	0.37 (0.10 , 1.55)	0.14	0.39 (0.08 , 1.32)	0.09

Table S4. Interaction between Flu in Trimester 3 and Antibiotic Use during Pregnancy on Odds of ASD

	OR (95% CI)	P-value	OR (95% CI)	P-value
Flu Trim 3	0.46 (0.03 , 2.31)	0.46	0.55 (0.03 , 2.84)	0.57
Antibiotic during Pregnancy	1.43 (0.88 , 2.46)	0.17	1.28 (0.76 , 2.30)	0.37
Child sex: male	---		3.05 (1.98 , 4.84)	0.00
Maternal education: HS graduation or below	---		0.80 (0.53 , 1.19)	0.26
Marital status: nor married	---		1.03 (0.67 , 1.58)	0.91
Maternal age (years)	---		1.03 (1.00 , 1.07)	0.04
Maternal BMI (ref: not overweight)	---			
Overweight	---		1.15 (0.70 , 1.86)	0.57
Obese	---		1.56 (0.96 , 2.52)	0.07
Diabetes/Gestational Diabetes	---		1.51 (0.88 , 2.51)	0.12
Pre-eclampsia	---		0.70 (0.34 , 1.32)	0.31
Flu Trim 3*Antibiotic during Pregnancy	2.66 (0.44 , 51.29)	0.37	2.73 (0.44 , 53.12)	0.37

Table S5. Interaction between Fever in Overall Pregnancy and Antibiotic Use during Pregnancy on Odds of ASD

	OR (95% CI)	P-value	OR (95% CI)	P-value
Fever Overall Pregnancy	1.05 (0.16 , 3.80)	0.95	1.13 (0.17 , 4.24)	0.87
Antibiotic during Pregnancy	1.51 (0.92 , 2.64)	0.12	1.35 (0.79 , 2.47)	0.30
Child sex: male	---		3.03 (1.97 , 4.81)	0.00
Maternal education: HS graduation or below	---		0.80 (0.54 , 1.21)	0.29
Marital status: nor married	---		1.03 (0.68 , 1.59)	0.89
Maternal age (years)	---		1.03 (1.00 , 1.07)	0.04
Maternal BMI (ref: not overweight)	---			
Overweight	---		1.15 (0.70 , 1.86)	0.58
Obese	---		1.53 (0.94 , 2.46)	0.08
Diabetes/Gestational Diabetes	---		1.51 (0.88 , 2.50)	0.12
Pre-eclampsia	---		0.70 (0.33 , 1.31)	0.30
Fever Overall Pregnancy *Antibiotic during Pregnancy	1.22 (0.29 , 8.40)	0.81	1.34 (0.31 , 9.36)	0.73

Table S6. Interaction between Fever in Trimester 1 and Antibiotic Use during Pregnancy on Odds of ASD

	OR (95% CI)	P-value	OR (95% CI)	P-value
Fever Trim 1	1.20 (0.07 , 6.21)	0.86	1.22 (0.07 , 6.53)	0.85
Antibiotic during Pregnancy	1.57 (0.97 , 2.70)	0.08	1.42 (0.84 , 2.54)	0.21
Child sex: male	---		3.04 (1.97 , 4.82)	0.00
Maternal education: HS graduation or below	---		0.79 (0.53 , 1.19)	0.26
Marital status: nor married	---		1.03 (0.68 , 1.60)	0.88
Maternal age (years)	---		1.03 (1.00 , 1.07)	0.04
Maternal BMI (ref: not overweight)	---			
Overweight	---		1.14 (0.69 , 1.85)	0.60
Obese	---		1.54 (0.95 , 2.48)	0.08
Diabetes/Gestational Diabetes	---		1.51 (0.88 , 2.49)	0.12
Pre-eclampsia	---		0.70 (0.34 , 1.32)	0.31
Fever Trim 1*Antibiotic during Pregnancy	0.76 (0.09 , 16.23)	0.82	0.92 (0.10 , 20.06)	0.95

Table S7. Interaction between Fever in Trimester 3 and Antibiotic Use during Pregnancy on Odds of ASD

	OR (95% CI)	P-value	OR (95% CI)	P-value
Fever Trim 3	1.60 (0.09 , 8.42)	0.65	1.68 (0.09 , 9.36)	0.63
Antibiotic during Pregnancy	1.57 (0.97 , 2.70)	0.08	1.42 (0.84 , 2.54)	0.21
Child sex: male	---		3.05 (1.98 , 4.83)	0.00
Maternal education: HS graduation or below	---		0.80 (0.54 , 1.20)	0.28
Marital status: nor married	---		1.03 (0.67 , 1.58)	0.91
Maternal age (years)	---		1.03 (1.00 , 1.07)	0.04
Maternal BMI (ref: not overweight)	---			
Overweight	---		1.14 (0.69 , 1.84)	0.60
Obese	---		1.53 (0.94 , 2.46)	0.08
Diabetes/Gestational Diabetes	---		1.51 (0.88 , 2.49)	0.12
Pre-eclampsia	---		0.71 (0.34 , 1.33)	0.32
Fever Trim 3*Antibiotic during Pregnancy	0.78 (0.11 , 15.82)	0.83	0.89 (0.12 , 18.55)	0.92

Table S8. Interaction between Genitourinary Infection and Antibiotic Use during Pregnancy on Odds of ASD

	OR (95% CI)	P-value	OR (95% CI)	P-value
Genitourinary Infection	0.61 (0.17 , 1.73)	0.39	0.64 (0.18 , 1.85)	0.44
Antibiotic during Pregnancy	1.51 (0.87 , 2.81)	0.17	1.27 (0.71 , 2.45)	0.45
Child sex: male	---		3.04 (1.98 , 4.82)	0.00
Maternal education: HS graduation or below	---		0.79 (0.53 , 1.19)	0.26
Marital status: nor married	---		1.04 (0.68 , 1.61)	0.86
Maternal age (years)	---		1.03 (1.00 , 1.07)	0.05
Maternal BMI (ref: not overweight)	---			
Overweight	---		1.14 (0.70 , 1.85)	0.59
Obese	---		1.57 (0.97 , 2.53)	0.06
Diabetes/Gestational Diabetes	---		1.50 (0.87 , 2.49)	0.13
Pre-eclampsia	---		0.70 (0.33 , 1.31)	0.29
Genitourinary Infection*Antibiotic during Pregnancy	1.32 (0.43 , 5.01)	0.65	1.35 (0.42 , 5.21)	0.64

Table S9. Interaction between any Maternal Immune Activation and Antibiotic Use during Pregnancy on Odds of ASD

	OR (95% CI)	P-value	OR (95% CI)	P-value
Any Maternal Immune Activation	0.91 (0.35 , 2.36)	0.84	0.81 (0.30 , 2.16)	0.67
Antibiotic during Pregnancy	1.55 (0.79 , 3.41)	0.24	1.17 (0.58 , 2.63)	0.67
Child sex: male	---		2.92 (1.88 , 4.69)	0.00
Maternal education: HS graduation or below	---		0.73 (0.49 , 1.11)	0.14
Marital status: nor married	---		1.08 (0.70 , 1.70)	0.73
Maternal age (years)	---		1.03 (1.00 , 1.06)	0.08
Maternal BMI (ref: not overweight)	---			
Overweight	---		1.20 (0.72 , 1.96)	0.47
Obese	---		1.59 (0.96 , 2.59)	0.07
Diabetes/Gestational Diabetes	---		1.37 (0.77 , 2.33)	0.26
Pre-eclampsia	---		0.59 (0.26 , 1.16)	0.16
Any Maternal Immune Activation*Antibiotic during Pregnancy	0.96 (0.34 , 2.7)	0.94	1.24 (0.42 , 3.69)	0.70

CHAPTER 6: DISCUSSION

This dissertation took a tripartite approach to studying the gastrointestinal (GI) system/gut in ASD (Figure 1).

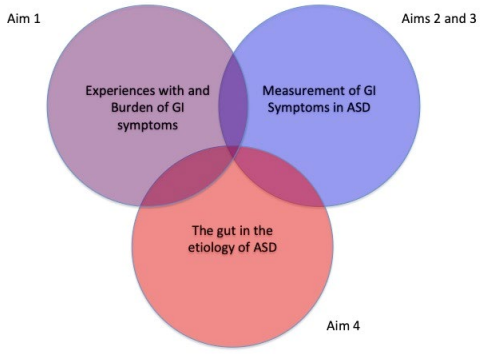

Figure 1. Tripartite Approach to the Gastrointestinal System/Gut in ASD

In Chapter 2 (Aim 1), the first study of this dissertation, we summarized the core themes that emerged from a dozen qualitative interviews with parents of children with ASD and co-occurring GI symptoms. These interviews were useful in helping derive the item pool for the ASD-GIRBI (Aim 3). We identified a number of indicators of GI symptoms in children who may not otherwise self-report experiencing GI symptoms. A limitation, however, is that these indicators may differ by child and may also not be specific to GI symptoms. Therefore, the consideration of a large number of indicators (e.g. poor sleep, irritability, aggression, weird postures) may be necessary to screen for GI symptoms in individuals with ASD, with follow-up for more comprehensive evaluation for GI disorders/pathology.

For some children in our study, indicators of severe GI distress included aggressive or violent behavior. As greater attention is paid to the management of crises in ASD, it will be critical to consider the role of medical symptoms in creating crisis situations[10,11]. Further, the strong link between GI symptoms and behaviors such as aggression and irritability is a reminder that these problem behaviors are often responses to stressors (e.g. GI distress and pain) rather than inherent

features of ASD[12,13]. Behavioral as well as clinical interventions need to consider that GI symptoms contribute to a portion of problematic behaviors, and that resolution of these behaviors may require treating the underlying medical issue.

The qualitative interviews also highlighted the significant toll that GI symptoms take on children with ASD as well as their families. Children with GI symptoms face difficulties attending and staying in class, participating in social or extracurricular activities, and experienced significant pain and distress due to their GI symptoms. Families described challenges to the overall wellbeing of the household, and in particular the overall temperament and stress level of families, and the ability for the family to leave the house or eat out at restaurants. Parents also described financial challenges associated with GI symptoms. It is important to remember how common these GI symptoms are for individuals with ASD when we consider the burden the wellbeing of the child and family. In Aim 1, we found that the median frequency of GI symptoms across the studies was 46.8%, meaning nearly half of individuals with ASD experience at least one GI symptom, though the range is wide and that number could be lower or much higher.

As we consider this burden, it is crucial to recognize the obstacles individuals in ASD face in having these symptoms recognized, evaluated, and treated. As discussed above, GI symptoms may be difficult to detect in individuals with ASD, especially in people who are non- or hypo-verbal or have cognitive impairments. Assuming that these GI symptoms do get recognized either by the individual with ASD or a parent/caregiver, there remain significant challenges.

The last major theme that emerged from the qualitative interviews was the negative experience that families tended to have when seeking healthcare for their child with ASD and GI symptoms. Challenges including long wait times between making an appointment and seeing a physician and financial costs and difficulties navigating the insurance system. Further, parents felt that medical offices were difficult places to bring their child with ASD, due to the loud, bright environment and the long wait times. Blood draws, which are often used for the assessment of various conditions, can be very difficult for individuals with ASD, and one parent in our study noted they would have to 'gas' their child to get blood work done.

A very troubling finding was that parents felt that the physician attributed the child's GI symptoms to autism and therefore their children did not receive the same evaluation, as would a child without ASD. In line with this, one parent stated that they wished they hadn't told the provider about their child having ASD. Diagnostic overshadowing, a process in which physical symptoms are inappropriately attributed to mental illness, is a reality for individuals with ASD, other developmental disability, and mental illnesses more broadly, and contribute to worse care[7,14].

The negative experiences and frustrations that parents have in seeking treatment for their child's GI symptoms likely contributes to the increasing popularity of complementary and alternative medicines/treatments (CAMs)[15]. In fact, one parent in our qualitative study described that among parent circles, CAMs such as bowel cleansing, are promoted given the long wait times in being able to see physician for their child's GI distress. This is expected and understandable, but also concerning given the lack of data on not only effectiveness of CAMs but also safety.

Research is urgently needed to study CAMs in general and in particular how they influence GI symptoms.

The next main goal of this dissertation work was to improve the measurement of GI symptoms in epidemiologic studies of ASD. In Chapter 3 (Aim 2), we reviewed studies of ASD dating from 1980 that reported GI symptoms, in order to understand the approaches to assessing GI symptoms in this population. As expected, we identified a wide range of tools that are used to ascertain GI symptoms. Most studies relied on questionnaires given to parents, while some studies used medical records to identify GI diagnoses, and a smaller portion of studies used symptom or stool diaries to prospectively measure GI symptoms. While there were several GI questionnaires designed specifically for the ASD population, at the time of the review none had been psychometrically assessed and we had no information on the reliability or validity of any of these tools. We found a very wide range (4.2 to 96.8%) of participants to have GI symptoms across the studies, with variability for symptom-specific estimates as well. Further, the frequency of specific GI symptoms across studies was associated with the assessment tool. For example, reflux symptoms were highest in studies that used medical records or claims data, likely because it's one of the GI disorders that are difficult to observe, and usually rely on physician examination or for someone to self-report symptoms such as "burning".

After having reviewed these studies of ASD, we were better able to identify gaps in current assessment tools. An important theme emerged: we need more standardized, reliable, and valid tools to estimate GI symptom prevalence since because current estimates are so varied across epidemiologic studies and because these symptom estimates are associated with the

measurement approach. This is critical given that GI symptoms appear to be one of the most common comorbid conditions in ASD and can significantly affect someone's quality of life[1,2]. In addition, the gut is quickly emerging as a possible risk factor for the development of ASD and comorbid conditions. The autism field desperately needs valid tools for assessing GI issues in both epidemiologic and clinical settings.

The review work led to the development and validation of a parent-report screener for GI symptoms, which we termed the ASD Gastrointestinal and Related Behaviors Inventory (ASD-GIRBI), described in Chapter 4. The ASD-GIRBI included items from two existing tools (the Autism Treatment Network GI Inventory[3] and the Brief Autism Mealtime Behaviors Inventory[4]) as well as new items. Critically, this item pool was more comprehensive and therefore had stronger content validity compared to prior tools, as it included GI symptoms, mealtime and dietary behaviors, as well as less specific behaviors that could reflect GI distress in a non-verbal or hypo-verbal child with ASD. We found the ASD-GIRBI to be an internally sound, i.e. reliable, tool for assessing the presence of GI symptoms and related behaviors. A 35-item screener with seven factors emerged from exploratory factor analysis: Factor 1: constipation & pain during bowel movements; Factor 2: doesn't want food at times; Factor 3: Particular with foods; Factor 4: abdominal pain/vomiting/gassiness/diarrhea; Factor 5: incontinence/soiling/wetting the bed; Factor 6: aggressive/disruptive at mealtimes; Factor 7: Other behaviors). Two factors on the screener (Factors 1 and 4, comprising of 13 items) detected parent-report of any GI disorder, constipation, and reflux with very high sensitivity, although the other 22 items on the questionnaire provide contextual information in research and clinical studies.

There were some limitations to our GIRBI development and evaluation. First, because of insufficient sample size, children ages 3-5 were not included in the psychometric analysis. Therefore, our results apply only to the group of 6-17 years old. Further, no adults with ASD were included in this study. Follow-up work will focus on developing an adult-version of this tool, which may be different from children. Second, we did not carry out item response theory analyses, so we do not know which of the 13 items among Factors 1 and 4 are particular important for detecting GI symptoms. Future work will include these analyses. And lastly, we did not have a gold-standard measure of GI symptoms in this population. While another recently validated ASD GI tool did have physician diagnosis, rather than parent-report diagnosis of GI symptoms[5], this also has limitations, as a patient's subjective experience of having GI symptoms may not be captured by a physician, especially if the patients is non- or hypo-verbal[6].

Challenges associated with medical testing in individuals with ASD also complicate the evaluation of these patients. The negative experiences parents had with providers described in Chapter 2 support this possibility. Parents reported that physicians did not take their child's GI symptoms as seriously because they assumed the symptoms to simply be part of their autism presentation. Diagnostic overshadowing is unfortunately common in ASD, as well as developmental disabilities and mental illnesses more broadly[7]. However, ASD-specific issues such as communication impairments, sensory issues, and severe food aversions seriously complicate this issue. Short parent-report screeners for individuals with ASD, such as the ASD-GIRBI, may improve the recognition of GI symptoms in both epidemiologic and clinical studies.

However, this tool is not particularly specific, and like with any screener, false positives need to be ruled out with further evaluation. Future work on this tool may improve the specificity.

Referring back to the orientating figure for this dissertation (Figure 1), the third goal for this study was to assess the role of the gut as a risk factor for the development of ASD. Prior research has implicated dysbiosis of the gut microbiome and dysregulation of the immune system in ASD. Animal and human studies have demonstrated that maternal immune activation and early-life infections increase the risk for ASD. Our growing understanding of the role of the gut microbiome in regulating the immune system has led to research demonstrating that in a mouse model, the maternal gut microbiota influences whether exposure to maternal immune activation in utero increases the risk of neurodevelopmental abnormalities and ASD-like symptoms in the offspring[8,9]. In Chapter 5 (Aim 4), using the Boston Birth Cohort, a prospective enriched-risk birth cohort of mother-newborn pairs enrolled and followed at the Boston Medical Center, we examined the interaction between maternal immune activation and antibiotic use in pregnancy on the risk of ASD in the offspring. In concordance with the animal literature, we show for the first time that antibiotic use in pregnancy appears to block the association between maternal immune activation, specifically flu in the second trimester, and risk of ASD in the child. In other words, flu in the second trimester was only a risk factor for ASD in the child in pregnant women who did not receive an antibiotic. This result remained after adjusting for a number of confounders, including C-section and preterm delivery; however, our study was underpowered and needs replication.

The findings from Chapter 5 are consistent with the strong evidence that the gut is a critical component in the development of neuropsychiatric disorders, given the animal literature linking maternal immune activation with maternal gut microbiome and fetal neurodevelopment. If the findings from this and other studies are replicated, the implications are profound. The conditions under which MIA increases the risk of neuropsychiatric conditions are unclear. This research highlights a potential modifying factor. Because the gut microbiome is malleable across the life course, including during pregnancy and in early-life, it is an attractive target for intervention. As we better understand what particular microbes protect against the negative sequelae of MIA, we may be able to develop therapies and ultimately decrease the incidence of ASD and other neuropsychiatric disorders such as schizophrenia. This work is preliminary but promising, and emphasizes the critical need for further studies of the gut microbiome and immune system.

A limitation of this dissertation overall is that most of the work was done in children. While our literature review of GI measurement approaches focused on all individuals, only 5 of those studies (5.7%) included individuals greater than 18 years of age. The opportunity to pilot our GI questionnaire, the ASD GIRBI, in a registry of families with a child with ASD was convenient, and the tool should indeed be piloted separately in children and adults. Future work on this questionnaire will involve adults with ASD. Because our qualitative study was in part designed to help us derived the item pool for the ASD GIRBI, we asked families to report on GI symptoms in their child with ASD at some point in childhood. We did have three families that had a child with ASD 18 years or older, but in future work we will also focus on recruitment of adults with ASD. Lastly, while Chapter 5 was focused on the etiology of ASD, we recognize the

importance of supporting individuals with ASD after the diagnosis, and not just preventing ASD and related disabilities.

The ultimate goal of this dissertation was to improve the detection of GI symptoms in individuals with ASD, assess the role of the gut as a causal risk factor for the incidence of ASD, and to decrease burden that GI symptoms place on individuals with ASD and their families, by shedding light on this issue. However, this work has implications for other developmental disabilities and mental illnesses more broadly. Gastrointestinal symptoms and other medical comorbidities are common in psychiatric disorders, and like in the case of ASD, can emerge for a number of reasons[16,17]. Accurate measurement of GI symptoms is particularly relevant to other developmental/intellectual disabilities or psychiatric conditions in which a person may not be able to self-report, such as during a serious episode of mental illness or in a person with dementia[18]. GI symptoms can take a toll on the wellness of any individual, and in particular individuals with a co-occurring psychiatric disorder. Individuals with mental illness also have negative experiences with the health care system and face disparities in access to quality health care, in part due to the complexity of their health conditions and also because of diagnostic overshadowing[7]. On a more hopeful note however, the gut likely plays an important role in the incidence, presentation, and course of mental illness broadly, and holds promise as a window for intervention[19,20]. The future of gut-brain research is exciting, has the potential to be a missing link in our understanding of psychopathology, and may help us in developing preventions and treatments for mental illness.

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APPENDICES

JOHNS HOPKINS
UNIVERSITY

Department of Mental Health
Bloomberg School of Public Health
624 N. Broadway / Baltimore, MD 21205-1999
(410) 955-3910 / FAX (410) 955-9088

Calliope Holingue, MPH
139 N Decker Ave
Baltimore, MD 21224

January 9, 2019

RE: Request to include previously published paper in final dissertation

Dear Calliope,

The Department of Mental Health Committee on PhD Program Requirements has approved your December 9th request to include in your Dissertation a first-authored manuscript published prior to completion of your Preliminary Oral Exams. This decision was made based on the following criteria:

1. Per a letter from Dr. Fallin, dated December 16, the **manuscript meets the standard for inclusion** in the Dissertation. Dr. Fallin stated: "Calliope conceived the question, sought advice on how to do the data collection, created a team to advise her, performed the searches, abstract and paper reviews, and data analyses, and wrote the prose of the paper."

2. The inclusion of this manuscript d**oes not add new aims nor substantially change the overall objectives** of the original Dissertation proposal. The content of this manuscript was used in the background of the Dissertation proposal and the manuscript could have easily been part of the originally developed dissertation plan.

This letter, along with your original request and the supporting letter from Dr. Fallin, will go into your academic file. We understand that you are still collecting data for the paper that you had originally intended to be your third manuscript and hope that you will be able to complete that part of the study at a later date.

We wish you the best of luck,

Sincerely,

Judith Bass, PhD MPH
Associate Professor and Vice Chair for Education
Department of Mental Health
Johns Hopkins Bloomberg School of Public Health
Email: jbass1@jhu.edu

Curriculum Vitae

Calliope Holingue, MPH, PhD

PERSONAL DATA

Business Address
Department of Mental Health
Johns Hopkins Bloomberg School of Public Health
Hampton House Office 798, 624 N. Broadway,
Baltimore MD 21205
choling1@jhu.edu, (443) 691-1125

EDUCATION AND TRAINING

Ph.D., Mental Health (May 2019)
Johns Hopkins University Bloomberg School of Public Health, Department of Mental Health

M.P.H., Epidemiology/Biostatistics (May 2015)
University of California, Berkeley

B.A., Double Major in Public Health and Molecular and Cell Biology (May 2013)
University of California, Berkeley

FELLOWSHIPS, GRANTS & AWARDS

- Gordis Teaching Fellowship, Johns Hopkins University for Course 'Mental Health and the Gut' Spring 2019
- Best Trainee Clinical Talk, Sleep and Circadian Research Day, Baltimore, MD, June 2018. 'Actigraphic Sleep and Functional Decline in Older Men'.
- Johns Hopkins Bloomberg School of Public Health Paul V. Lemkau Scholarship Fund Award (2018)
- Center for Innovative Care in Aging Pilot Grant, Johns Hopkins School of Nursing, 'Screening for Dementia in Older Adults with Intellectual/Developmental Disabilities', Role: Co-PI, Funding Amount: $2,499.00 (2018)
- Wendy Klag Center for Autism & Developmental Disabilities Student Grant Award, Johns Hopkins Bloomberg School of Public Health, 'Microbiome Composition and Structure of Children with Autism Spectrum Disorder', Role: Principle Investigator, Funding Amount: $13,938 (2017)
- NIEHS Environmental Epidemiology of Autism Research Network (EEARN) Meeting Travel Scholarship (2017)
- Johns Hopkins Bloomberg School of Public Health Dr. Ali Kawi Scholarship Award (2017)
- Johns Hopkins Bloomberg School of Public Health Student Assembly Student Recognition

Award (2016)
- National Institute of Mental Health Psychiatric Epidemiology Training Program (Fall 2015-current)
- Rheumatology Research Foundation Health Professional Research Preceptorship (2015)
- UC Berkeley School of Public Health Block Grant Award, Amount: $10,000 (2014-2015)

Assisted with Successful Grant Submissions
- 2016-2017 Drug Dependence Epidemiology Training Program Renewal (Drs. Brion Maher and Renee Johnson)
- 2015-2016 Psychiatric Epidemiology Training Program Renewal (Dr. Peter Zandi)
- 2015-2016 Pilot Grant to assess patterns of DNA methylation as biomarkers of response to treatment of Rheumatoid Arthritis (Dr. Lindsey Criswell)

RESEARCH AND PROFESSIONAL EXPERIENCE

Doctoral student in Mental Health (September 2015-present)
Department of Mental Health, Bloomberg School of Public Health, Johns Hopkins University
Dissertation (Advisor: Dr. M. Daniele Fallin)
- Aim 1: Gastrointestinal symptoms in autism spectrum disorder: A review of the literature on ascertainment and prevalence
- Aim 2: Development of a parent-report questionnaire assessing gastrointestinal symptoms among in children with Autism Spectrum Disorder.
- Aim 3: Interaction between maternal immune activation and antibiotic use during pregnancy on risk of autism spectrum disorder, in an enriched-risk prospective birth cohort study.
- Aim 4: Qualitative study of family experiences with having a child with ASD and gastrointestinal symptoms

Researcher at The Center for START (Systemic, Therapeutic, Assessment, Resources & Treatment) Services (March 2017-present)
- Experiences with the Mental Health Service System of Family Caregivers of Individuals with an Intellectual/Developmental Disability referred to START
- Dementia screening of older adults with intellectual/developmental disabilities
- Mental and physical health of older individuals with intellectual/developmental disabilities
- Mental and physical health of adults with Down Syndrome
- Polypharmacy among individuals with intellectual/developmental disabilities
- State-wide needs assessments

Intern/researcher in Genetic Epidemiology and Genomics (May 2014-May 2015)
Genetic Epidemiology and Genomics Laboratory (Dr. Lisa Barcellos), UC Berkeley
- Cleaned and analyzed genotype and methylation data as part of analyses of rheumatoid arthritis susceptibility and phenotype.
- Explored genetic determinants of cognitive impairment in MS by creating a genetic risk

score, based on literature review of SNPs associated with cognitive impairment in other diseases.

- Carried out statistical analyses to examine the association between population density and multiple sclerosis (MS) susceptibility.

Researcher in Evaluation (Jan. 2013-Dec. 2013)

On-campus/Online Professional M.P.H. Degree Program, UC Berkeley

- Supported UC Berkeley's first online degree program by helping to ensure accreditation and meet university requirements as well as assisting with internal evaluation process.
- Collected data by writing a focus group protocol, designing course evaluations and faculty/graduate student instructor surveys, conducting faculty/staff meetings and analyzing student performance data.
- Designed evaluations, collected and analyzed qualitative and quantitative data and communicated findings in person and in written reports.

PEER-REVIEWED PUBLICATIONS (*first author)

1. Kalb, L. G., Stapp, E. K., Ballard, E. D., **Holingue, C**., Keefer, A., & Riley, A. (2019). Trends in psychiatric emergency department visits among youth and young adults in the US. *Pediatrics*, *143*(4), e20182192.
2. Schneider, K.E., **Holingue, C.,** Roth, K.B., Eaton, W. (2019). 'Enduring Mental Health in the Baltimore Epidemiologic Catchment Area Follow-Up Study'. *Social Psychiatry and Psychiatric Epidemiology. https://doi.org/10.1007/s00127-019-01676-z*
3. Kalb, L., Stapp, E., Ballard, E., **Holingue, C.,** Keefer, A., Riley, A. (2019). 'National Trends in Psychiatric Emergency Department Visits among Youth and Young Adults'. *Pediatrics*. 10.1542/peds.2018-2192
4. Owusu, J.T., Wennberg, A.M.V., **Holingue, C.B**., Tzuang, M., Abeson, K.D., Spira, A.P. (2018). 'Napping Characteristics and Cognitive Performance in Older Adults'. *Journal of the American Geriatrics Society*. https://doi.org/10.1002/gps.4991
5. **Holingue, C.*,** Owusu, J., Feder, K.A., Spira, A.P. (2018). 'Sleep duration and C-reactive protein: Associations among pregnant and non-pregnant women.' *Journal of Reproductive Immunology.* doi: 10.1016/j.jri.2018.05.003
6. **Holingue, C.*,** Wennberg, A., Berger, S., Polotsky, V. Y., & Spira, A. P. (2018). 'Disturbed Sleep and Diabetes: A Potential Nexus of Dementia Risk.' *Metabolism*. doi: 10.1016/j.metabol.2018.01.021
7. Mok, A., Rhead, B., **Holingue, C**., Shao, X., Quach, H.L., Quach, D., Sinclair, E., Graf, J., Imboden, J., Link, T. and Harrison, R., (2017). 'Hypomethylation of CYP2E1 and DUSP22 promoters associated with disease activity and erosive disease among rheumatoid arthritis patients.' *Arthritis & Rheumatology*. doi: 10.1002/art.40408
8. Goetzel, R., Roemer, E.C., **Holingue, C.**, Fallin, M.D., McCleary, K., Eaton, W., Agnew, J., Azocar, F., Ballard, D., Bartlett, J., Braga, M., Conway, H., Crighton, K.A., Frank, R., Jinnett, K., Keller-Greene, D., Rauch, S.M., Safeer, R., Saporito, D., Schill, A., Shern, D., Strecher, V., Wald, P., Wang, P., Mattingly, R. (2017). 'Mental Health in the Workplace: A Call to Action

Proceedings from the Mental Health in the Workplace: Public Health Summit.' *Journal of Occupational and Environmental Medicine.* doi: 10.1097/JOM.0000000000001271

9. **Holingue, C**.*, Newill, C., Lee, L. C., Pasricha, P. J., & Daniele Fallin, M. (2017). 'Gastrointestinal symptoms in autism spectrum disorder: A review of the literature on ascertainment and prevalence.' *Autism Research.* doi: 10.1002/aur.1854
10. George, M., **Holingue, C.,** Briggs, F., Shao, X., Bellesis, K., Whitmer, R., … Barcellos, L. (2016). 'Feasibility study for remote assessment of cognitive function in multiple sclerosis.' Journal of Neurology and Neuromedicine, 1(8), 10–18. http://www.jneurology.com/
11. Rhead, B., **Holingue, C.*,** Cole, M., Shao, X., Quach, H. L., Quach, D., Shah, K., Sinclair, E., Graf, J. Link, T., Harrison, R., Rahmani, E., Halperin, E., Wang, W., Firestein, G.S., Barcellos, L.F., Criswell, L.A. (2016). 'Rheumatoid arthritis naïve T cells share hypermethylation sites with synoviocytes.' *Arthritis & Rheumatology.* doi: 10.1002/art.39952

MANUSCRIPTS UNDER REVIEW/REVISION

1. **Holingue, C.,** Kalb, L., Klein, A., Beasley, K. 'Experiences with the Mental Health Service System of Family Caregivers of Individuals with an Intellectual/Developmental Disability referred to START.' Under Revision at Intellectual and Developmental Disabilities.

MANUSCRIPTS IN PREPARATION

1. **Holingue C.,** Owusu J.T., Yaffe K., Stone K.L., Rebok G.W., Ancoli-Israel S., Spira AP. 'Actigraphic Sleep and Functional Decline in Older Men.'
2. **Holingue, C**., Patti. M.A., Croen, L.A., Hertz-Picciotto, I., Pandey, J., Newschaffer, C.J., Fallin, M.D. Prebiotic and Probiotic Consumption during Pregnancy and Autism Observational Scale for Infants (AOSI) Score at 12-Months in the Early Autism Risk Longitudinal Investigation (EARLI).'
3. **Holingue, C.,** Patti, M.A., Truong, C., Schneider, K, Eaton, W. 'Systematic Review of Panic Disorder in Population-Based Cohort Studies.'
4. Patti, M.A., Truong, C., Schneider, K, **Holingue, C.,** Eaton, W. 'Systematic Review of Simple Phobia Disorder in Population-Based Cohort Studies.'
5. Truong, C., Patti, M.A., **Holingue, C.,** Schneider, K, Eaton, W. 'Systematic Review of Major Depressive Disorder in Population-Based Cohort Studies.'
6. Park, B.Y., Volk, H.E., **Holingue, C**., Jones, K., Ashwood, P., Windham, G.C., Lurmann, F., Alexeeff, S.E., Kharrazi, M., Pearl, M., Van de Water, J., Croen, L. 'Prenatal Air Pollution Exposure, Maternal Immune Markers, and Risk of Autism Spectrum Disorder and Intellectual Disability in the Early Markers for Autism (EMA) Study.'
7. Patti, M., **Holingue, C.,** Kalb., L., Volk, H. 'Developmental Disabilities and Medical Conditions: Characterizing Prevalence of CAM Use.'
8. **Holingue, C.,** Spira. A., Mueller, N. 'Relationship between sleep duration and gut microbiota composition and diversity in the American Gut Project.'
9. **Holingue, C.,** Fallin. D., Mueller, N. 'Relationship between autism spectrum disorder and gut microbiota composition and diversity in the American Gut Project.'
10. **Holingue, C.,** Ingram, W., Guglielmi, V., Samuels, J., Nestadt, G. 'Gestational Diabetes is Associated with Exacerbation of Obsessive-Compulsive Symptoms during Pregnancy.'
11. Zandi, P., **Holingue, C.,** Colder Carras, M., Riehm, K., Rojo Wisar, D., Ingram, W., Roth, K.,

Nestadt, P., Musliner, K., Haroz, E., Eaton, W. 'The Future of Psychiatric Epidemiology.'

BOOK REVIEWS

1. **Holingue, C.** The American Journal of Psychiatry (2018). Review of: Scott, Kate M., et al., eds. *Mental Disorders around the World: Facts and Figures from the World Mental Health Surveys*. Cambridge University Press, 2018. https://doi.org/10.1176/appi.ajp.2018.18050506

BOOK CHAPTERS

1. Eaton, William W., et al. "Hopes and Challenges Moving Forward." *Public mental health* (2019).

GREY LITERATURE

1. Ingram, W., **Holingue**, C., Wilcox, H., Fallin, D. (2018). 'Looking to Make Lasting Change: Building a Mental Health Graduate Student Network.' Public Health Chronicle, Journal for Johns Hopkins University Bloomberg School of Public Health. https://www.wendymarieingram.com/wp-content/uploads/2018/07/Ingram_Holingue_MHGradNetwork_PublicHealthChronicle-1.pdf
2. Beasley, J.B., Klein, A., Caoili, A., **Holingue, C.B.** The Center for START Services. 'State of Georgia MH/IDD Service System Analysis'. February 12, 2018
3. Fallin, M.D., Martinez, T., …**Holingue, C.,** Branch, F. Johns Hopkins University. 'Task Force on Student Mental Health and Wellbeing'. February 2018.
4. **Holingue, C.B.,** Roemer, E.C., Goetzel, R., Fallin, M.D. The Luv u Project. 'Advancing Mental Health Through Action. Summary notes from the Mental Health in the Workplace: A Public Health Summit, held at Johns Hopkins University Bloomberg School of Public Health, on October 20, 2016.' May 2017.

SERVICE & LEADERSHIP

1. Co-founder & President, JHSPH Mental Health Graduate Student Network (Spring 2018-present)
2. Co-leader of Special Interest Group 'Gastrointestinal Issues in Autism Spectrum Disorder', 2018 International Society for Autism Research, Rotterdam, May 2018 & 2019
3. Mental Health Student Group Vice President (2016-2017), President (2017-2018)
4. Member of Johns Hopkins University Task Force for Mental Health and Well-Being (Summer 2016-December 2018)
5. President, Amnesty International UC Berkeley Chapter (2011-2012)

CONFERENCES AND INVITED PRESENTATIONS

1. **Holingue C.,** Owusu J.T., Yaffe K., Stone K.L., Rebok G.W., Ancoli-Israel S., Spira AP. 'Actigraphic Sleep and Functional Decline in Older Men", Sleep and Circadian Research Day, Baltimore, MD, June 2018.
2. **Holingue C.,** Owusu J.T., Yaffe K., Stone K.L., Rebok G.W., Ancoli-Israel S., Spira AP.

'Actigraphic Sleep and Functional Decline in Older Men", ID number 698, Session Number O25. SLEEP, 32nd Annual Meeting of the Associated Professional Sleep Societies, Baltimore, MD, June 2018.

3. **Holingue, C.** 'Gastrointestinal Issues in Autism Spectrum Disorder.' Special Interest Group, 2018 International Society for Autism Research, Rotterdam, Netherlands, May 2018.
4. **Holingue, C.** Kalb, L., Beasley, J., Klein, A., Hinton, J., Charlot, J. 'START Family Caregivers mental health service experiences', START National Training Institute, Boston, Massachusetts, May 2018.
5. **Holingue, C.** "The gastrointestinal system and gut microbiome in Autism Spectrum Disorders'. Departmental Seminar Talk, Department of Mental Health, Johns Hopkins University School of Public Health, Baltimore, MD, March 2018.

CONFERENCE ABSTRACTS

1. Weigle, K., Charlot, L., **Holingue, C.** 'Mental and Physical Health Conditions of Individuals with Intellectual and Developmental Disabilities (IDD) with and without Down Syndrome (DS).' To be presented at Gatlinburg Conference on Research and Theory in Intellectual and Developmental Disabilities to be presented April 2019, San Antonio, Texas.
2. **Holingue C.,** Owusu J.T., Yaffe K., Stone K.L., Rebok G.W., Ancoli-Israel S., Spira AP. 'Actigraphic Sleep and Functional Decline in Older Men", ID number 698, Session Number O25. SLEEP, 32nd Annual Meeting of the Associated Professional Sleep Societies, Baltimore, MD, June 2018.
3. Kalb, L., Charlot, L. Beasley, J., Caoili, A., Klein, A., Holingue, C., Hinton, J. 'National Center for START Services Research Committee', START National Training Institute, Boston, MA, 2018.
4. **Holingue C.,** Owusu J.T., Yaffe K., Stone K.L., Rebok G.W., Ancoli-Israel S., Spira AP. 'Actigraphic Sleep and Functional Decline in Older Men", 11th Annual Research on Aging Showcase, Baltimore, MD, April 20, 2018.
5. Wise, E., **Holingue, C.**, Klein, A., Caoili, A., Charlot, L. Beasley, J. 'Psychiatric Outcomes in Older Adults with Intellectual and Developmental Disabilities (IDD).' 11th Annual Research on Aging Showcase, Baltimore, MD, April 20, 2018.
6. Schneider, K., Roth, K., **Holingue, C.,** Musci, R. 'Racial Differences in the Effect of Adverse Childhood Experiences (ACES) on Adult Alcohol Consumption: a Latent Class Analysis', ID number 446. Research Society on Alcoholism, San Diego, CA, June 2018.
7. **Holingue C.,** Owusu J.T., Yaffe K., Stone K.L., Rebok G.W., Ancoli-Israel S., Spira AP. 'Actigraphic Sleep and Functional Decline in Older Men', ID number 321, Session Number P38. SLEEP, 32nd Annual Meeting of the Associated Professional Sleep Societies, Baltimore, MD, June 2018.
8. **Holingue, C.,** Mueller, N., Fallin, M.D. 'Similarity of Citizen-Science American Gut Project to Published ASD Studies on Gut Microbiota', ID 28398. 2018 International Society for Autism Research, Rotterdam, Netherlands, May 2018.
9. Goetzel, R., Roemer, E.C., **Holingue, C.**, Fallin, M.D., McCleary K., Eaton, W., Agnew, J., Azocar, F., Ballard, D., Bartlett, J., Braga, M., Conway, H., Crighton, K.A., Frank, R., Jinnett, K., Keller-Greene, D., Rauch, S.M., Safeer, R., Saporito, D., Schill, A., Shern, D., Strecher, V.,

Wald, P., Wang, P., Mattingly, R., 'Mental Health in the Workplace: A Call to Action.' 2nd International Symposium to Advance Total Worker Health, Natcher Conference Center on the campus of the National Institutes of Health, Bethesda, Maryland, May 2018.

10. Schneider, K., Roth, K., **Holingue, C. '**Racial Differences in the Effect of Adverse Childhood Experiences (ACES) on Adult Alcohol Consumption: A Latent Class Analysis.' Abstract 767. Poster to be presented at Research Society on Alcoholism, June 2018, San Diego, CA.
11. **Holingue, C.,** Feder, K., Owusu, J.T., Spira, A.P. 'Is the Association between Sleep and Inflammation Modified by Pregnancy?' Sleep & Circadian Rhythm Research Day, June 26th, 2017, Baltimore, MD.
12. Tzuang, M., **Holingue, C.,** Owusu, J.T., Parisi, J.M., Spira, A.P., Rebok, G.W. 'Does Cognitive Training Improve Sleep in Older Adults? Findings from the ACTIVE Memory Works.' Sleep & Circadian Rhythm Research Day, June 26th, 2017, Baltimore, MD.
13. Owusu, J.T., Wennberg, A., **Holingue, C.**, Tzuang, M., Abeson, K., Spira, A.P. 'Napping Characteristics and Cognitive Performance in Older Adults'. Sleep & Circadian Rhythm Research Day, June 26th, 2017, Baltimore, MD.
14. Schneider, K., **Holingue, C.,** Roth, K., Eaton, W. 'Predictors of Enduring Mental Health in the Baltimore Epidemiologic Catchment Area Study.' American Public Health Association (APHA) 2017 Annual Meeting & Expo, **Nov 4th, 2017**, Atlanta, GA.
15. Tzuang, M., **Holingue, C**., Spira, A. 'Sleep in Older Caregivers and Non-Caregivers: The National Health and Aging Trends Study.' Poster presented at the 21st IAGG World Congress of Gerontology and Geriatrics, **July 2017**, San Francisco, CA.
16. **Holingue, C.**, Newill, C., Lee, L., Pasricha, P., Fallin, M.D. 'Need for Valid, Reliable Gastrointestinal Symptoms Measurement Tool for Autism Spectrum Disorder, a Review of the Literature', Abstract 24476. 2017 International Meeting for Autism Research, **May 11, 2017,** San Francisco, CA.
17. **Holingue, C**., Croen, L. A., Hertz-Picciotto, I., Pandey, J., Newschaffer, C.J., Fallin, M.D. 'Prebiotic and Probiotic Consumption during Pregnancy and Autism Observational Scale for Infants (AOSI) Score at 12-Months in the Early Autism Risk Longitudinal Investigation (EARLI).' Poster presented at the 2016 Meeting of the International Meeting for Autism Research, **May 13, 2016,** Baltimore, MD.
18. **Holingue, C.**, Eaton, W. 'C-reactive protein is significantly associated with depressive symptoms in a nationally representative survey.' Poster presented at the 106th Annual Meeting of the American Psychopathological Association, **March 3, 2016,** New York City, New York.
19. Tzuang, M., **Holingue, C.**, Spira, A. 'Sleep in Older Caregivers and Non-Caregivers: Findings from the National Health and Aging Trends Study.' Poster presented at the **2016** Johns Hopkins University Sleep and Circadian Research Day, Baltimore.
20. **Holingue, C.** 'Proposed Research Project: Characterization of Gastrointestinal Symptoms and Feeding Behaviors in a Longitudinal Enriched-Risk Autism Cohort.' Poster presented at the **2015** "One Size Doesn't Fit All: Learning about Autism from Studies Big and Small" Wendy Klag Center for Autism symposium, Johns Hopkins University, Baltimore.
21. **Holingue, C**., Rhead, B., Cole, M., Shao, X., Quach, H., Quach, D., Sinclair, E., Graf, J., Link, T., Harrison, R., Chernitskiy, V., Wang, W., Firestein, G., Barcellos, L., Criswell, L. 'DNA

Methylation Changes Observed in Rheumatoid Arthritis Joint Tissue Are Detectable in CD4+ Naive T Cells from Peripheral Blood', Abstract 1610. Poster presented at the 2015 ACR/ARHP Annual Meeting, **November 2015**, San Francisco, CA.

22. Mok, S., **Holingue, C**., Rhead, B., Cole, M., Shao, X., Quach, H., Quach, D., Sinclair, E., Graf, J., Link, T., Harrison, R., Chernitskiy, V., Wang, W., Firestein, G., Barcellos, L., Criswell, L. 'DNA Methylation Profiling of Rheumatoid Arthritis Peripheral Blood Identifies Hypermethylation of TRIM69 Promoter Region in CD4+ T Cells Associated with Disease Activity', Abstract 1620. Poster presented at the 2015 ACR/ARHP Annual Meeting, **November 2015,** San Francisco, CA.
23. **Holingue C.,** George M., Schaefer C., Bernstein A., Whitmer R., Barcellos L. 'Examining Associations Between Multiple Sclerosis Cognitive Impairment and Genes Previously Associated with Cognitive Decline in Other Disease', Abstract 753T. Poster presented at the 64th Annual Meeting of the American Society of Human Genetics, **October 21, 2014,** San Diego, CA.
24. Rhead B., **Holingue C.,** Cole M., Shao X., Quach H., Quach D., Barcellos F., Criswell L. 'DNA Methylation Profiles That Distinguish Rheumatoid Arthritis from Osteoarthritis in Fibroblast-Like Synoviocytes Can Be Detected in Immune Cells from Peripheral Blood', Abstract 460T. Poster presented at the 64th Annual Meeting of the American Society of Human Genetics, **October 21, 2014**, San Diego, CA.

TEACHING & COURSE DEVELOPMENT

Gordis Teaching Fellowship (awarded in Fall 2018 for Spring 2019 course)
Mental Health and the Gut, Johns Hopkins University

Preparing Future Faculty Teaching Academy Certificate Program (awarded Fall 2018)

Course Design and Instruction, 6-week course (Fall 2018)
Mental Health and the Gut - for Special Opportunities for Undergraduate Learning
Krieger School of Arts & Sciences, Johns Hopkins University

Teaching Assistant (Fall 2018)
The Public Health Approach to Psychopathology - Online and Blended sections (Adam Spira),
Bloomberg School of Public Health, Johns Hopkins University

Course Design and Instruction, 2-day course (Summer 2018)
Mental Health and the Gut
Department of Mental Health, Bloomberg School of Public Health, Johns Hopkins University

Course Design and Instruction, 1-day course (Summer 2018)

Mind-Altering Microbes – for Miracles of Modern Medicine Program
Johns Hopkins University

Teaching Assistant (Spring 2018)
Public Health Approaches in Autism and Developmental Disabilities (Li-Ching Lee and M. Daniele Fallin), Bloomberg School of Public Health, Johns Hopkins University

Course Design and Instruction- 2-week course (Winter 2017-2018 Intersession)
Mental Health and the Gut, Johns Hopkins University

Teaching Assistant (Fall 2017)
The Public Health Approach to Psychopathology (Adam Spira), Bloomberg School of Public Health, Johns Hopkins University

Teaching Assistant in Designing Online Course and Redesigning Blended Course (Spring-Fall 2017)
The Public Health Approach to Psychopathology (Adam Spira), Bloomberg School of Public Health, Johns Hopkins University

Teaching Assistant (Summer 2016)
Public Health Approaches in Autism and Developmental Disabilities (Li-Ching Lee and M. Daniele Fallin), Bloomberg School of Public Health, Johns Hopkins University

Head Graduate Student Instructor (Spring 2015)
Introduction to Epidemiology and Human Disease (Lisa Barcellos and Mahasin Mujahid), School of Public Health, UC Berkeley

Graduate Student Instructor (Fall 2014)
Graduate Epidemiologic Methods (Arthur Reingold), School of Public Health, UC Berkeley

Graduate Student Instructor (Spring 2014)
Undergraduate Physiology (Daniela Kaufer), Integrative Biology Department, UC Berkeley

AD-HOC REVIEWER EXPERIENCE
Autism Research, Autism, Metabolism, Developmental Psychobiology

MEDIA COVERAGE OF WORK

- Fallin, D., & Holingue, C. (2017). Autism studies hampered by lack of reliable test for gut problems. Retrieved from https://spectrumnews.org/opinion/viewpoint/autism-studies-hampered-lack-reliable-test-gut-problems/